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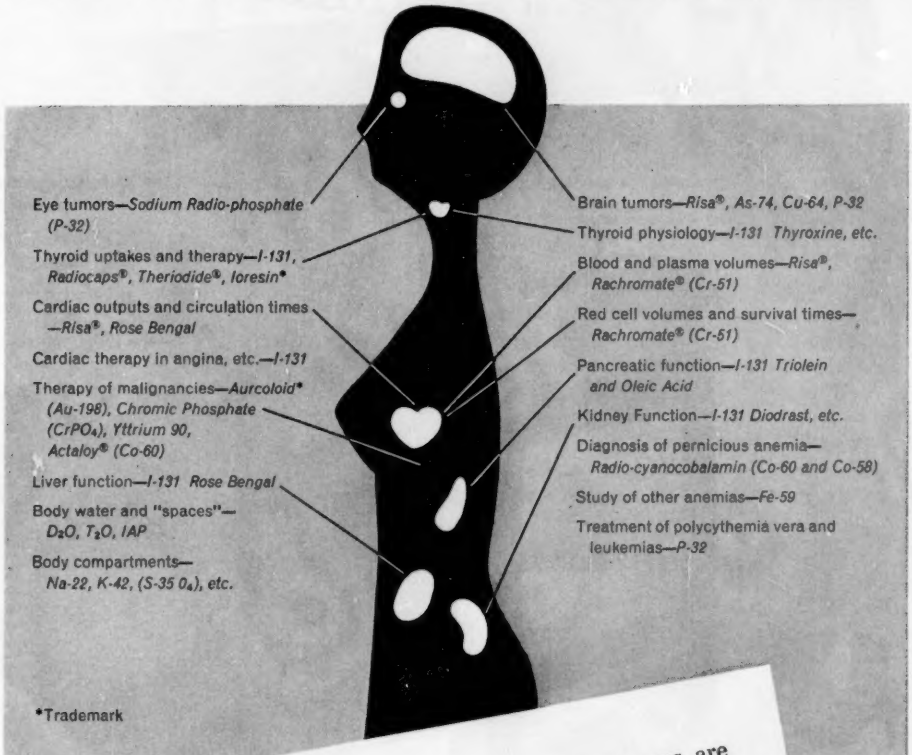
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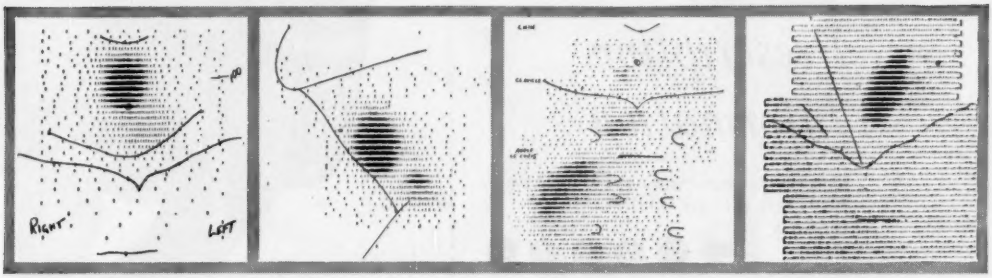

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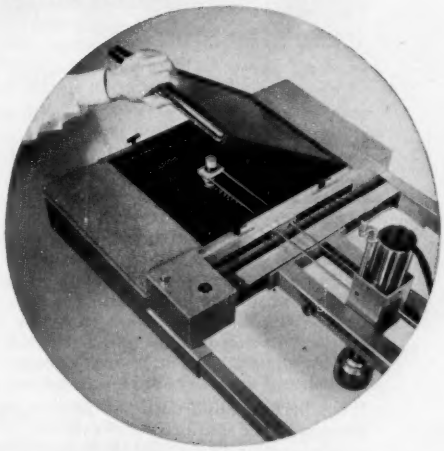

Reference: David E. Kuhl, R. H. Chamberlain, John Hale and R. O. Gorson: A High-Contrast Photographic Recorder for Scintillation Counter Scanning. Radiology, pgs. 730-739, May, 1956.

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DIASTOLIC GALLOP SOUNDS, THE MECHANISM OF PRODUCTION [1, 2]

By PETER T. KUO,[3] TRUMAN G. SCHNABEL, JR.,[4] WILLIAM S. BLAKEMORE,[5] AND ARTHUR F. WHEREAT

(From the Edward B. Robinette Foundation, Medical Clinics, Hospital of the University of Pennsylvania, and The Harrison Department of Surgical Research of the School of Medicine, University of Pennsylvania, Philadelphia, Pa.)

(Submitted for publication November 5, 1956; accepted January 30, 1957)

In a study previously reported from this laboratory, the mechanism of gallop sounds was studied with the aid of the electrokymograph (1). A wave of rapid lateral movement of the left ventricular wall was repeatedly demonstrated in each of the 28 patients with hypertension and heart failure with either a protodiastolic or presystolic gallop sound. The gallop sound occurred during this wave and not after its completion. The abnormal left ventricular border kymogram disappeared with the regaining of compensation and subsidence of the gallop sound in these patients. It was felt that these observations supported the views of Potain (2), Ohm (3), Wolferth and Margolies (4), Orias and Braun-Menendez (5), and Mannheimer (6) that an impact or sudden stretching of the ventricular wall is the chief mechanism of production of the gallop sound.

On the other hand, Hirschfelder (7), Gibson (8), Thayer (9), Sewall (10), Lewis and Dock (11), Brady and Taubman (12), and Dock, Grandell, and Taubman (13) supported the theory of reflux closure of the atrio-ventricular valves as the mechanism for the production of the gallop sound on the basis of studies of apical impulse, jugular vein pressure pulse, left ventricular kymogram and ballistocardiogram.

The development of right and left heart catheterization (14, 15) and, particularly, the development of techniques to secure an accurate simultaneous recording of the atrial and ventricular pressure pulses (15) have made it possible to further the investigation of the origin of the gallop sound. In this study the temporal relationships of the gallop sound to the atrial, superior vena caval, and external jugular pulses of the same subject were determined. Apex cardiograms were also studied in this series of patients. The recording characteristics of the piezoelectric pick-up and the Lilly capacitance manometer employed in the study were compared.

MATERIAL AND METHODS

Two groups of patients were used in this investigation: a) Left heart catheterization was performed to evaluate 10 patients with chronic rheumatic mitral valvular disease for cardiac surgery. Clinical evidence of a predominant mitral insufficiency, and that of a complicating aortic lesion were indications for the pre-operative left heart study. b) Right heart catheterization was performed on 4 patients with signs and symptoms of severe right heart failure to help establish the etiological diagnosis of the heart disease.

Right heart catheterization was performed according to the technique of Cournand and Ranges (14) and by the use of a small double-lumen catheter. Pulmonary "capillary" pressure was recorded by the method described by Lagerlöf and Werkö (16) and by Hellems, Haynes, and Dexter (17). For left heart catheterization a technique developed by Schnabel, Blakemore, Kuo, and Langfeld (15) for the simultaneous recording of pressures in the left atrium and left ventricle was used. Atrial and ventricular pressures were recorded simultaneously using two Lilly capacitance manometers either through a No. 7 double lumen Cournand catheter or through two small plastic catheters (18). The time lag of the small catheter system was 0.002 second or less. The apex impulse was obtained by the use of a glycerine capsule end-piece attached alternately to a) a piezoelectric pick-up, and b) a Lilly capacitance manometer. A specially constructed multichannel Hathway recorder which includes a phonocardiographic channel was used for all the recordings in this study. All records were taken at a paper speed of 75 mm. per second.

[1] This study has been supported in part by Grant H-393 of the United States Public Health Service.

[2] Presented in part at a Symposium on Cardiovascular Sounds, Cincinnati, Ohio, Oct. 26, 1956.

[3] This work was done during the tenure of a Research Fellowship and the tenure of an Established Investigatorship of the American Heart Association.

[4] Markle Scholar in Medical Sciences.

[5] Scholar in Cancer Research.

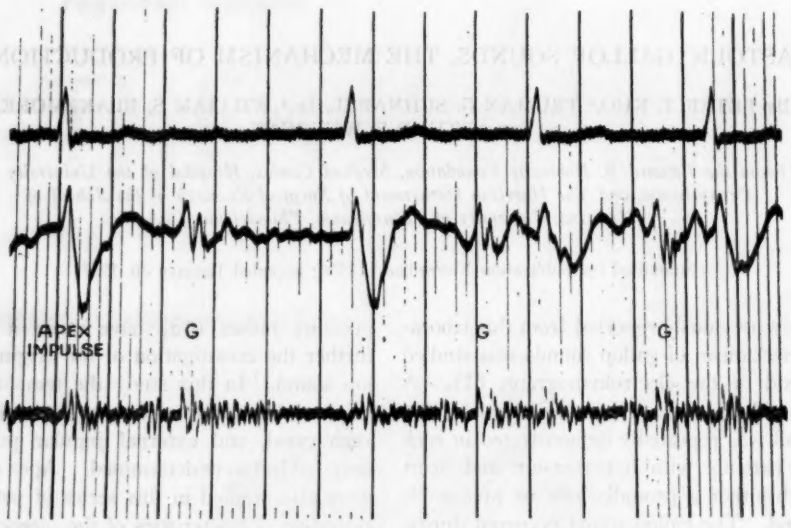

FIG. 1. APEX IMPULSE RECORDED WITH A PIEZOELECTRIC PICK-UP ON A PATIENT WITH MITRAL INSUFFICIENCY AND LOUD PROTODIASTOLIC GALLOP SOUND (G)

A sharp downward dip (inflection) following an initial upswing is recorded both during ventricular systole and at the time of the protodiastolic gallop sound.

RESULTS

Correlation of the apex impulse to the gallop sound

Apex impulses of this series of 14 patients with protodiastolic and presystolic gallop sounds were recorded both through a piezoelectric pick-up and through the amplifying system of the Lilly manometer. A typical example of the apical impulse obtained through a piezoelectric pick-up from a patient with a protodiastolic gallop is shown in Figure 1. In this case the apex impulse starts to move upward (outward) about 0.04 second before the onset of the gallop sounds (G in Figure

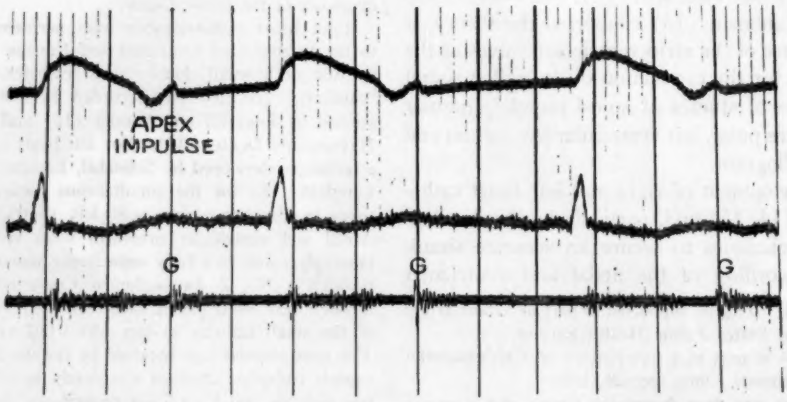

FIG. 2. APEX IMPULSE RECORDED WITH A LILLY CAPACITANCE MANOMETER ON A PATIENT WITH MITRAL INSUFFICIENCY AND A PROTODIASTOLIC SOUND (G)

Both the systolic and the diastolic out-thrusts are well sustained and they do not show the sharp inflections and other extraneous vibratory movements.

1). It rises sharply to the peak of outward thrust in 0.04 to 0.06 second, and is followed by a sharp downward dip (inflection). Similar sharp upward and downward swings in the apex cardiogram are seen with each apical thrust during ventricular systole. Finger pressures of varying abruptness and firmness were applied to the end-piece of the piezoelectric pulse wave pick-up to simulate apical out-thrusts. These maneuvers produced upward swings of varying sharpness not unlike the systolic and diastolic apex beats recorded in Figure 1. Each of the upward swings is followed by a sharp dip (inflection) of varying depth, which in turn is followed by a few after-vibrations.

Apex impulses were then taken on these patients by attaching the glycerine capsule end-piece to the Lilly manometer. The systolic and diastolic apical thrusts taken by this method are shown in Figure 2. In this tracing well-sustained systolic and diastolic apical out-thrusts are recorded. The extraneous movements are almost entirely eliminated, and an "inflection" wave is not seen either during ventricular systole or in protodiastole.

Correlation of the gallop sound with jugular vein pulse curve

The practices of using the jugular pulse curve to time various mechanical events of the cardiac cycle and of correlating these events to the onset of the gallop sounds were investigated by simultaneous recording of the right atrial and jugular vein pulse pressures in 4 patients with right heart failure and right-sided gallop sounds. The delay in the transmission of pulse wave from the mid-portion of the right atrium to the jugular vein at the level of the middle of the neck was found to range between 0.14 to 0.20 second in these patients.

In a patient with severe right heart failure and a right-sided presystolic gallop sound, the pulse pressures of the right atrium, superior vena cava, and external jugular vein were recorded sequentially with phonocardiogram and electrocardiogram. By selecting beats with approximately equal cycle lengths from the three tracings and then by carefully aligning the P waves in one tracing with the other two, we were able to obtain: 1) the respective time relationships between the "a" wave, as recorded with a catheter in the right atrium, in the superior vena cava, and in the external jugular vein, and the P wave in the electrocardiogram, and 2) the time relationships of these "a" waves obtained from different sites, with respect to one another. Figure 3 shows a progressive delay in the occurrence of the "a" wave in reference to the P wave in the electrocardiogram as one moves the catheter from the right atrium to the jugular vein. This delay may be as much as 0.14 to 0.20 second. For this reason the presystolic gallop which falls in good alignment with the peak of the right atrial "a" wave might well be related erroneously to some part of the "V" wave in the preceding cardiac cycle, if a jugular pulse curve were used for the purpose of timing.

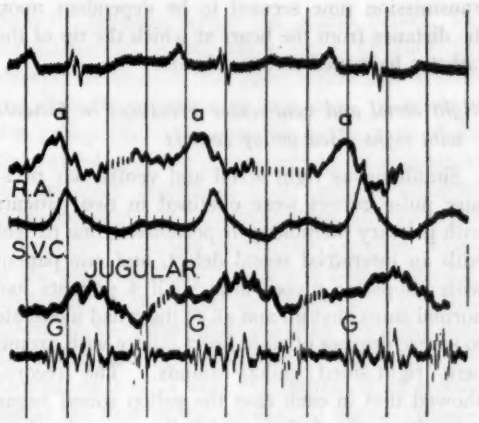

FIG. 3. TRACING ILLUSTRATES A PROGRESSIVE DELAY OF THE "a" WAVE WITH RESPECT TO THE P WAVE IN THE ELECTROCARDIOGRAM AS THE CARDIAC CATHETER IS WITHDRAWN FROM THE RIGHT ATRIUM TO THE JUGULAR VEIN

Correlation of the pulmonary "capillary" pressure with left-sided gallop

Both right and left heart catheterizations were performed in 3 of the 10 patients with left-sided gallop sounds and predominant mitral insufficiency. Successful wedging of the catheter in one of the smaller branches of the pulmonary artery was achieved in all 3 patients.

A slight delay in the transmission of the "a" and "V" waves (amounting to only 0.01 to 0.03 second) was observed in the pulmonary wedge pressures of these 3 patients compared with their respective left atrial pressure curves obtained subsequently by left heart catheterization or by direct puncture of the left heart chambers during cardiac surgery. The length of the pulse wave

transmission time seemed to be dependent upon the distance from the heart at which the tip of the catheter happened to be wedged.

Right atrial and ventricular pressures in patients with right-sided gallop sounds

Simultaneous right atrial and ventricular pressure pulse curves were obtained in two patients with primary pulmonary hypertension, one patient with an interatrial septal defect, and one patient with idiopathic myocarditis. All 4 patients had normal sinus rhythm and all of them had moderate to severe degrees of right heart failure with prominent right-sided gallop sounds. The records showed that in each case the gallop sound began near the peak of the atrial "a" wave. In these patients, the gallop sounds occurred while the right atrial pressure was 3 to 11 mm. Hg higher than that of the right ventricle. The highest atrioventricular pressure differential was observed in a patient with idiopathic myocarditis who had a P–R interval of 0.32 second and a summation gallop. Examples of the right heart pulse pressure studies are presented in Figures 4a and 4b, 5a and 5b. Figure 4a was obtained from patient L.A. who had a large interatrial septal defect with a predominant left to right shunt, congestive heart failure, and a loud gallop sound (G) as recorded in the phonocardiogram. The gallop was best heard and was easily felt at the region of the xyphoid cartilage. The tracing shows that the gallop sound occurs at the peak of the atrial "a" wave. Replotting of the right atrial and ventricular pressures of the second and third heart beat in Figure 4a on an equal and expanded scale and from the same base line (Figure 4b) gives a graphic demonstration of the relationship of the gallop sound to the intracardiac pressures. The atrial pressure was 8 to 9 mm. Hg higher than the ventricular pressure when the gallop sounds appeared. This pressure gradient across the tricuspid valve was not maintained to the point immediately prior to the rise of right ventricular pressure. No tricuspid stenosis was found during cardiac surgery.

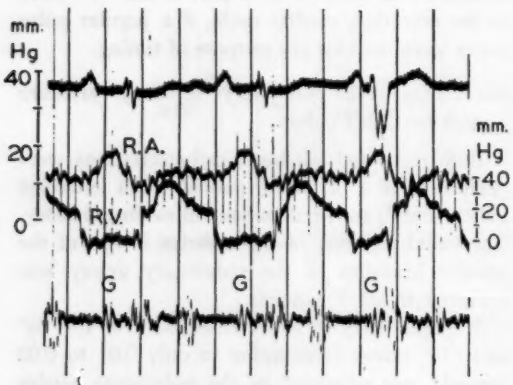

FIG. 4a. SIMULTANEOUS RIGHT ATRIAL AND VENTRICULAR PRESSURE CURVES OBTAINED FROM A PATIENT WITH INTERATRIAL SEPTAL DEFECT, RIGHT HEART FAILURE AND A RIGHT-SIDED GALLOP SOUND

The gallop (G) is recorded at the peak of atrial systole.

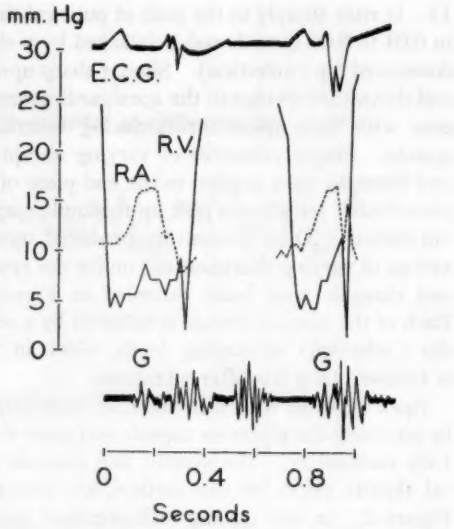

FIG. 4b. REPLOTTING THE ATRIAL AND VENTRICULAR PRESSURE CURVES OF THE SECOND AND THIRD HEART BEAT IN FIGURE 4a ON A COMMON BASE LINE AND ON THE SAME SCALE SHOWS THE RIGHT ATRIAL PRESSURE TO BE HIGHER THAN THAT OF THE RIGHT VENTRICLE FOR ABOUT 0.5 SECOND IN DURATION

The gallop sound (G) starts near the peak of the atrial "a" wave.

Figures 5a and 5b were made from patient M.H. who had idiopathic myocarditis. A routine right heart catheterization was performed on him 3 years ago. The findings obtained in that study were within the range of normal. In the interim the patient went into congestive heart failure and developed a loud right-sided summation gallop. He was readmitted to the hospital for study. Be-

cause of X-ray evidence of an enlarged left atrium and the obscure nature of the cardiac lesion, a left heart catheterization was done to rule out mitral valvular disease. Simultaneous left atrial and ventricular pressure measurements showed no diastolic gradient across the mitral valve. Recatheterization of the right heart (Figure 5a) showed elevation of the right ventricular systolic and diastolic pressures at this time. A summation gallop (G) was recorded at the peak of the right atrial pressure curve, which, on account of the markedly prolonged P–R interval, occurred in protodiastole. Coarse vibrations were recorded in both the atrial and ventricular pressure curves at the time the gallop sound was being recorded. Replotting of the atrial and ventricular pressure curves on the same scale and from the same base line shows a large atrio-ventricular pressure differential of 9 to 11 mm. Hg during atrial systole and at the time of the summation gallop (Figure 5b).

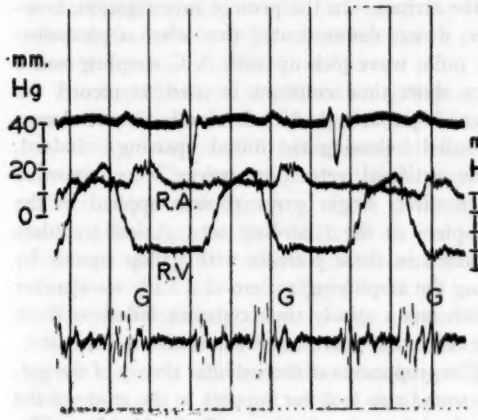

FIG. 5a. SIMULTANEOUS RIGHT ATRIAL AND VENTRICULAR PRESSURE CURVES OBTAINED FROM A PATIENT WITH IDIOPATHIC MYOCARDITIS, CONGESTIVE HEART FAILURE, AND A RIGHT-SIDED SUMMATION GALLOP (G)

Gallop sound G is recorded near the peak of the atrial "a" wave.

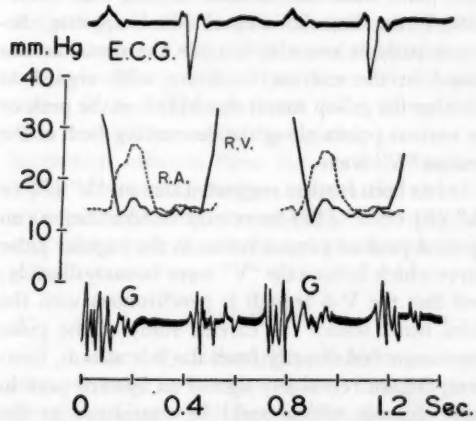

FIG. 5b. REPLOTTING OF THE ATRIAL AND VENTRICULAR PRESSURE CURVES IN FIGURE 5a ON A COMMON BASE LINE AND ON THE SAME SCALE SHOWS AN ELEVATION OF THE ATRIAL PRESSURE OVER THAT OF THE VENTRICULAR PRESSURE FOR ABOUT 0.4 SECOND

The summation gallop (G) occurs near the peak of the atrial "a" wave where the atrio-ventricular pressure differential is at its peak value.

Left atrial and ventricular pressure in patients with left-sided gallop sounds

Simultaneous recordings were obtained by left heart catheterization of the left atrial and left ventricular pressures through two separate catheters in 10 patients with mitral insufficiency and gallop sound. In 7 of the 10 patients a protodiastolic gallop was recorded while the left atrial pressure was 6 to 18 mm. Hg higher than that of the left ventricle. In three patients with presystolic gallop the sound was recorded when the left atrial pressure was 3 to 6 mm. Hg above the left ventricular pressure. The left heart pressure curves obtained from a patient, M.H., with mitral insufficiency are shown in Figure 6a. The left atrial pressure curve shows: 1) a small rise in early ventricular systole due most likely to the closure of the A-V valves, 2) a sharp rise in pressure during late ventricular systole due to mitral insufficiency, and 3) the timing of the gallop sound to the lower third of the descending limb of the sharp "V" wave. No vibration of any kind was recorded in the left atrial pressure curve either during or after the occurrence of the gallop sound. On the other hand, a marked increase in coarse vibrations is seen in the left ventricular pressure curves in early diastole. The diagnosis of mitral insufficiency was confirmed at operation and was subsequently proven at autopsy. When the left atrial and ventricular pressure curves are plotted from the same base line and on a common scale, it is clear that the left atrial pressure is 12 to 13 mm. Hg higher than the left ventricular pressure

FIG. 6a. SIMULTANEOUS LEFT ATRIAL AND VENTRICULAR PRESSURES OBTAINED FROM A PATIENT WITH MITRAL INSUFFICIENCY, HEART FAILURE, AND A PROTODIASTOLIC GALLOP SOUND (G)

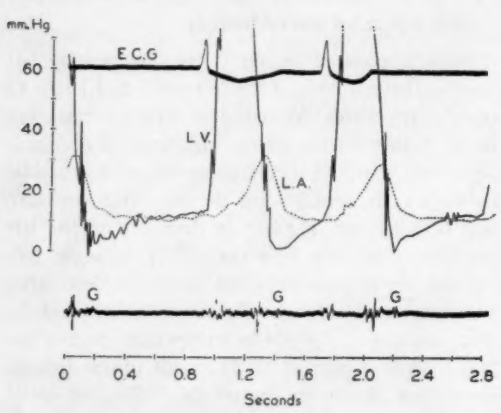

FIG. 6b. REPLOTTING OF THE PRESSURE CURVES IN FIGURE 6a ON A COMMON BASE LINE AND ON THE SAME SCALE SHOWS THE GALLOP SOUND (G) OCCURS AT THE LOWER THIRD OF THE DESCENDING LIMB OF THE EXAGGERATED "V" WAVE

The atrial pressure is higher than the ventricular pressure before, during and after the protodiastolic gallop sound.

at the moment the gallop sound occurs (Figure 6b).

DISCUSSION

Those investigators, who in their attempts to marshall evidence in support of the theory of reflux closing of the atrio-ventricular valves as the mechanism for the production of the gallop sound, have repeatedly emphasized the importance of apical impulse recordings in the study of patients with protodiastolic gallop (11–13). They have contended that the momentary retraction or the "sharp inflection" recorded in the tracing following the initial outward thrust is supportive of the assumption that the gallop sound is invariably accompanied by a reflux of blood in the direction of the atrium. In the present investigation, however, it was demonstrated that when a piezoelectric pulse wave pick-up with A–C coupling and a very short time constant is used to record the apical impulse, a sharp inflection is almost always recorded following the initial upswing. Indeed, these artificial retraction waves were recorded when direct finger pressure was applied to the end-piece of the recording set. Apical impulses obtained in these patients with gallop sounds by using the amplifying system of a Lilly manometer which has a steady time constant failed to show the retraction wave after the initial out-thrust.

The proponents of the valvular theory of the gallop sound also look for support in the study of the jugular venous pulsations (7, 8, 11–13). The demonstration of a delay of 0.14 to 0.20 second in the transmission of the venous pulse from the right atrium to the jugular vein clearly invalidates its use in an attempt to correlate gallop sound with certain events within the heart. The delay in venous pulse transmission time coupled with differences in the diastolic interval (the heart rate) between patients can also explain the controversies raised in the current literature with regard to whether the gallop sound should fall on the peak or on various points along the descending limb of the venous "V" wave.

It has been further suggested that an "h" (7) or "b" (8) or "f" (19) wave may be identified as an upward peak of venous reflux in the jugular pulse curve which follows the "V" wave in early diastole; and that the V-h trough is synchronous with the third heart sound. A careful study of the pulse wave recorded directly from the left atrium, however, did not reveal any sign of an upward peak in early diastole which could be considered as the possible origin of the so-called venous reflux wave observed in the jugular pulse curve (Figure 6a). In this connection it is pertinent to observe that when separate catheters are used to record the left atrial and left ventricular pressure curves, little or no extraneous vibration is recorded in the atrial curve while many coarse vibrations are

seen in the ventricular curve at the time of the occurrence of the gallop sound (Figure 6a). We are in agreement with the suggestion (20) that a "pure" jugular-venous pulse is difficult to obtain technically. It is more than likely that the wave of venous reflux observed in some patients with protodiastolic gallop rhythm is due to the inclusion in the venous pulse of certain parts of the carotid or aortic pulsations.

The hypothesis of a summation gallop as advanced by Wolferth and Margolies (4) has been repeatedly confirmed by numerous clinical observations. The large atrio-ventricular pressure differential recorded in one of the patients who had a minor grade A–V heart block and right-sided gallop further affirms the summation concept in the production of gallop sounds.

Comparative study of the wedge pressure and left atrial pressure curve obtained from the same patient either by left heart catheterization or by direct puncture of the left atrium during cardiac surgery shows a comparatively short delay in the pulse wave transmission time. Hence, in the absence of a true left atrial pressure, the pulmonary capillary pressure (wedge) is best suited for timing certain left-sided intracardiac events.

The simultaneous measurement of atrial and ventricular pressures on the side of the heart where the gallop sound originates shows that at the time of occurrence of the gallop sound the atrial pressure is invariably higher than the ventricular pressure. It is unlikely that the A–V valves could close and vibrate with the existing pressure relationships between these two cardiac chambers. These observations further support the view that an impact or sudden stretching of the ventricular wall is the basic mechanism of production of the gallop sounds.

SUMMARY

1. A sharp dip following an initial upswing is often recorded in the apex beat by using a piezoelectric pick-up which has a short time constant. The artificial wave of apical retraction can be eliminated by the use of the amplifying system of the Lilly manometer.

2. The temporal relationships of the atrial, vena caval, and jugular "a" wave to the P wave in the electrocardiogram and between one another have been studied and compared. The data indicate that a delay in the venous pulse wave transmission from the atrium to jugular vein may amount to 0.14 to 0.20 second.

3. Comparing the pulmonary wedge pressure with the left atrial pressure obtained in the same patient, a small delay in the pulse wave transmission time is demonstrated. The delay is only about 0.01 to 0.03 second.

4. A venous reflux wave is not demonstrated in the pressure curves taken directly either in the right or left atrium at the time the gallop sound occurs. Therefore, the upward deflection which comes immediately after the "V" wave in the jugular venous pulse is probably due to the inclusion in the venous pulse of some components of the arterial pulse.

5. Simultaneous atrial and ventricular pressure measurements made on the side of the heart where the gallop sound originates show that the atrial pressure is invariably higher than the ventricular pressure at the time of the gallop sound. These observations further support the view that a sudden impact or stretching of the ventricle is the basic mechanism involved in the production of gallop sounds.

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RADIOACTIVE IRON METABOLISM AND ERYTHROCYTE SURVIVAL STUDIES OF THE MECHANISM OF THE ANEMIA ASSOCIATED WITH RHEUMATOID ARTHRITIS [1]

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Anemia of moderate severity occurs frequently in rheumatoid arthritis patients (1). These patients show abnormalities of iron metabolism such as low serum iron concentration and rapid loss of injected iron from the plasma (1–4). Abnormalities of red cell survival have been described (5–7), while studies of heme pigment excretion products have not shown evidence of increased hemoglobin catabolism (8). The role of these disturbances in the genesis of the anemia in arthritic patients is not clearly understood.

The present study was undertaken to define the role of changes in rates of red cell production and destruction in the pathogenesis of the anemia occurring in rheumatoid arthritis patients, and to clarify the effect of disturbances of iron metabolism on red cell production in these patients. An abstract of this work has been published previously (9).

MATERIALS AND METHODS

A. Clinical material

1. Patients. The patients studied had active rheumatoid arthritis and were hospitalized for at least the first three weeks of the study. Cases were selected without regard for the presence or degree of anemia. Those with evidence of active bleeding or past history of significant blood loss were excluded from the study. Study cases received only conservative therapy during the period of this investigation, consisting of bed rest, physiotherapy, and salicylates. None of the study cases were known to have received previous blood transfusions.

2. Controls. The control subjects were normal healthy adults who worked full time and had no past or present evidence of chronic disease or of blood loss. These subjects were selected in an age group comparable to that of patients studied. Controls were not screened for normal serum iron or hemoglobin levels. Healthy male medical students served as blood donors and recipients of blood transfusions from arthritis patients for red cell survival studies.

B. Plan of study

Patients were fasted from 8 P.M. the evening preceding the study. From 8:30 to 9:30 A.M. blood was withdrawn for routine hematology and for serum iron determination. Immediately following this, Evans' blue dye and plasma-bound radioactive iron were successively injected intravenously using gravimetrically calibrated syringes (± 0.3 per cent). Samples of venous blood were taken from the opposite arm through an indwelling needle at 10-minute intervals for the first 30 minutes and at 30-minute intervals during the subsequent 3 to 5 hours. Over the next 14 to 21 days samples of venous blood were taken for measurement of red cell uptake of the injected radioiron. Stools were collected for measurement of fecal urobilinogen excretion. After completion of these studies, patients found suitable for red cell survival studies were given a blood transfusion and had samples of venous blood removed every 7 to 14 days during the following 120 to 140 days.

C. Radioiron studies

1. Isotope. Fe-59 of high specific activity (1 to 5 millicuries per milligram) was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tennessee, in the form of ferric chloride. The isotope was converted chemically to ferric ammonium citrate and the pH of the solution adjusted to 6.8 to 7.0. The latter was then diluted to an appropriate volume and sterilized by autoclaving.

2. Radioactivity detection techniques. All radioactivity assays were performed with gamma scintillation

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counting equipment, using thallium-activated, sodium iodide crystals. Liquid samples were counted in a 1-inch thick, well-type crystal. The counting efficiency was approximately 4 to 5 per cent. All samples were counted under conditions of constant geometry and sufficient counts recorded to give a counting error of less than 2 per cent. Standard iron solutions were counted in alternation with samples to correct for instrument variation and to eliminate the necessity for decay correction. External body monitoring was done using a 1-inch by 1-inch sodium iodide crystal housed in a lead-shielded portable probe which could be placed in contact with the skin over various organs (*e.g.*, spleen, liver). Activity was expressed as "counts per minute."

3. Plasma Fe-59 removal rate (clearance). Heparinized plasma, freshly drawn from a normal donor, was used as a source of iron-binding globulin. One-tenth to 0.3 ml. of the Fe-59 ammonium citrate was added to 12 to 15 ml. of plasma. Less than one microgram of inorganic iron was added to each ml. of plasma and the plasma was then incubated at 37° C. for 30 minutes to insure complete binding of the added iron to available iron binding protein. The total radioactivity injected ranged from 5 to 10 microcuries, and the total quantity of inorganic iron injected ranged from 3 to 12 micrograms. Standards were prepared by appropriate saline dilution of an aliquot of the original Fe-59 transferrin solution used for injection. Plasma samples obtained during the 3 to 5 hours after injection of the Fe-59 labelled plasma were assayed for radioactivity. The rate of removal of Fe-59 from the plasma was computed from these data using two different models (Figure 1).

Model A: This computation was done by the method of Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence (10). In this model, the Fe-59 clearance is assumed to be an exponential function of time. A straight line was fitted by the method of least squares to the data ($y = \log$ Fe-59 activity [net counts per minute], $X =$ time [hours]). The Fe-59 space was determined from the computed value of this line at zero time. The time required for the removal of 50 per cent of the Fe-59 activity was computed from these data.

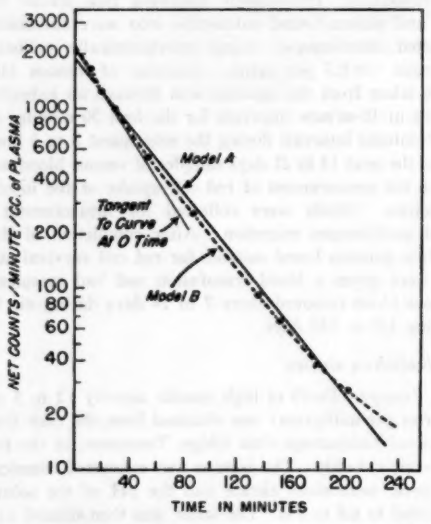

FIG. 1. GRAPHIC PRESENTATION OF THE TWO MODELS USED FOR COMPUTATION OF THE RATE OF REMOVAL OF RADIOIRON FROM THE PLASMA

Model B: In many instances these values when plotted on semi-log paper showed what appeared to be a slight but definite curvature. Thus a second degree curve of the form ($\log$ counts $= a + bt + ct^2$) was fitted by the method of least squares. The half-time of Fe-59 removal and the Fe-59 space were computed from a tangent to this curve at time zero by the method of Dornhorst (11).

4. Plasma iron turnover rate. This was calculated by the method of Huff and his co-workers (10), using the following formula: Ln 2/half time plasma Fe-59 clearance [hours]) × Fe-59 space [ml.] × serum iron concentration [mgm. per ml.] × 24 = plasma iron turnover rate [mgm. per day]).

5. Red cell uptake of Fe-59. Four ml. of whole blood was hemolyzed with saponin and assayed for radioactivity. A hematocrit reading was obtained on an aliquot. The activity of each sample expressed as counts per minute per ml. of packed red cells, multiplied by the red cell mass of the subject, gave the total activity present in the red cell mass. This value, divided by the injected activity, gave the per cent red cell uptake of injected Fe-59.

6. Erythrocyte iron turnover rate. This was calculated by multiplying plasma iron turnover rate (No. 4) by the maximum per cent red cell Fe-59 uptake (No. 5), and expressed as mgm. per day.

7. External body monitoring. Body surveys were made at 30 to 60-minute intervals on the day of injection of the Fe-59 and at 1 to 3-day intervals thereafter. The detecting probe was placed directly on the skin over indelibly marked organ sites. The gross counting rate was corrected by subtracting the counting rate over the thigh to give the net counting rate at each organ site. An Fe-59 standard was counted each time a body survey was done and the counts were then corrected for decay to the activity at Day One.

8. The significance of the difference between means (p) was computed by the method of Aspin and Welch (12).

D. Serum iron concentration

Serum iron was determined by the method of Kitzes, Elvehjem, and Schuette (13), using an Evelyn photoelectric colorimeter and orthophenanthrolene as an indicator. Samples were tested in duplicate and any showing hemolysis were discarded.

E. Plasma, red cell and total blood volume measurement

Plasma volume was measured simultaneously with Evans' blue dye and transferrin bound Fe-59. The method of Gibson and Evelyn (14) was used for Evans'

blue plasma volume determination. Ten ml. of a 0.1 per cent solution of dye was injected intravenously. The concentration of dye in plasma samples was measured with a Beckman spectrophotometer, using a one-cm. light path, or with the micro attachment of the Evelyn colorimeter. Hemolyzed samples were discarded. The concentration of dye and the radioactivity present in the 10-, 20-, and 30-minute samples were plotted against time on semi-log paper and the curves extrapolated back to zero time. The concentration of dye and the radioactivity present at zero time were used for calculation of plasma volume. The values for plasma volume as measured by Evans' blue dye and transferrin bound Fe-59 agreed closely, the mean difference being 3 per cent. The lower of the two values was used for calculation of red cell mass.

Total blood volume and red cell volume were calculated from plasma volume and the mean of hematocrits of the blank, 10-, 20-, and 30-minute samples. Hematocrits were done by the method of Wintrobe (15) and spun at 2,000 G for 60 minutes. The observed hematocrit was corrected for trapped plasma (16) and by a factor of 0.91 to correct for the ratio of total body to venous hematocrit (17).

F. Erythrocyte survival studies

The technique of Ashby, as modified by Ebert and Emerson (18), was used. Type O blood was transfused into type A recipients or subtype N blood was transfused into subtype M recipients. The number of inagglutinable cells remaining in the recipient's circulation was determined at 7 to 14-day intervals until none remained. The recipient's cells were agglutinated by the use of potent, dried, powdered rabbit antisera (Lederle). The sample for counting was diluted 1:200 (0.1 ml. to 20 ml.) in sterile normal saline. A red cell count was performed on this suspension. Then approximately 0.2 ml. of this dilution was added to a small amount (10 to 20 mgm.) of the dry antiserum and shaken until all antiserum was dissolved. The tube was then spun at 500 r.p.m. for five minutes and the cells were resuspended by gentle flicking of the tube. This was repeated once and then the cell suspension was transferred to a counting chamber. A minimum of 500 unagglutinated cells was counted in each chamber when possible, and a minimum of 1,000 cells was counted for each sample. All counts were performed by the same technician, in duplicate. Black counts were performed on two separate occasions on all recipients, and only those subjects with inagglutinable cell counts of less than 20,000 per cubic mm. were used for this study. Blood was infused into recipients within 18 hours after collection into plastic bags (Fenwal) containing A.C.D. anticoagulant solution. The blood was given in the form of a replacement transfusion, a phlebotomy of 500 to 800 ml., being followed immediately by an infusion of 500 to 1,000 ml. of donor blood. Thereafter, the blank count was subtracted from the observed gross inagglutinable cell count to give the net inagglutinable cell count per cubic mm. of blood. The net inagglutinable cell count (y axis) was then plotted against time in days (x axis) on rectangular coordinate paper. A curve was then drawn through these points.

NoQ curves were constructed by the technique of DeGowin, Ellis, Sheets, Hamilton, and Janney (19), by the use of the following formula: $NoQ = n/(1 - [t/T])$. Where n = observed net inagglutinable cell count, t = time in days when the sample was drawn. However, rather than use an assumed value of 120 days for T as was done by the above authors, the observed intercept of the original curve with the time axis was used as T. NoQ values were calculated for every observed value of n and plotted on semi-log paper against time. These points described what appeared to be an exponential function. The rate of red cell destruction was calculated from these data. The percentage of red cells lost per day as a result of linear decay was computed from 1/T and the percentage of cells lost per day as a result of exponential decay was computed from ln 2/time in days required to remove 50 per cent of the donor cells by exponential decay alone (NoQ curve). The sum of these two values gave the total rate of red cell loss (per cent per day).

G. Fecal urobilinogen

This was measured by the technique of Schwartz, Sborov, and Watson (20), using an Evelyn photoelectric colorimeter calibrated with Pontacyl dye standards. All stools were collected for four-day periods and kept in opaque bottles under continuous refrigeration. Hemolytic indices were calculated by the method of Miller, Singer, and Dameshek (21).

H. Miscellaneous

Body surface area was determined from height and weight by the use of the DuBois nomogram (22). Hemoglobin was measured as oxyhemoglobin with an Evelyn photoelectric colorimeter which was calibrated on the basis of Van Slyke gas analysis. Sedimentation rates were determined by the Rourke-Ernstene method and expressed as the corrected blood sedimentation index (B.S.I.) in mm. per minute (23). Reticulocytes were stained with brilliant cresyl blue and counted by the dry method. Total circulating hemoglobin was determined by the product of the red cell mass and M.C.H.C. per 100. The total circulating hemoglobin iron was calculated from the total circulating hemoglobin using the value of 0.339 per cent as the iron content of hemoglobin (24).

RESULTS

A total of 42 patients and 10 control subjects was studied. The mean age of the patients was 53.3 years, and of the controls, 51.0 years. The subjects are listed by number in Table I.

A. Hematology (Table I)

The patients studied had a lower mean hemoglobin concentration (11.7 Gm. per cent) than the

controls (14.9 Gm. per cent). The female patients' mean hemoglobin (11.3) was lower than the male patients' mean hemoglobin (12.3). Likewise the female controls' mean hemoglobin (13.1) was lower than the male controls' mean hemoglobin (15.0). The values for hematocrit showed similar differences, the patients having a lower mean value (38.2 per cent) than the controls (43.1 per cent). The red cell counts also showed similar differences, the patients having lower mean values (4.50×10^6 per cubic mm.) than controls (4.76).

Computation of red cell indices revealed that the patients had a lower average Mean Corpuscular Hemoglobin (27.7 γγ) than the controls (29.5 γγ), a slightly lower average Mean Corpuscular Volume (89.9 cubic micra) than controls

TABLE I

No.	Age	WT. (Kg.)	SURF. AREA (Sq. M.)	HGB (Gms. %)	HCT %	RBC x 10^6/cm.	BLOOD SED. INDEX mm/min	RETICS %	PLASMA VOL. ml.		RBC Vol. ml.	TOTAL BLOOD VOL. ml.	PLASMA IRON TURNOVER RATE (mg./day)		RBC UPTAKE	
									EVANS BLUE	Fe^{59}			MOD. A	MOD. B	%	DAYS
I. FEMALE PATIENTS																
1	62	49.7	1.49	11.80	38.5	4.34	1.0	1.6	—	2610	1309	3919	25.82	27.28	88.6	11
2	45	61.0	1.58	11.32	37.5	4.54	1.0	1.9	3153	3356	1484	4637	20.89	21.83	96.5	10
3	71	46.3	1.41	12.16	39.0	4.65	1.1	1.0	2112	2244	1054	3166	36.78	39.41	88.4	11
4	54	45.7	1.40	11.00	35.5	4.22	0.85	2.6	3184	3260	1358	4542	35.72	38.41	86.7	10
5	47	53.0	1.61	9.98	34.5	4.37	1.45	0.6	2803	2819	1230	4033	20.08	21.78	88.1	9
6	44	55.9	1.56	10.45	33.6	3.78	1.65	0.3	2741	2717	1267	3984	10.40	10.80	85.4	7
7	62	49.4	1.49	11.67	35.8	4.26	1.1	0.5	2587	2643	1179	3760	21.99	27.96	82.1	18
8	65	61.4	1.66	11.0	35.2	3.98	0.4	1.4	2964	2955	1266	4221	24.83	25.40	93.9	9
9	67	60.0	1.65	10.75	34.6	3.54	0.6	1.3	2047	2011	1131	3142	14.99	17.45	96.1	13
10	39	95.6	1.94	12.59	40.0	4.14	1.4	1.0	2970	3029	1476	4446	24.81	25.89	87.9	17
11	47	48.6	1.53	9.30	30.0	4.25	—	0.9	3061	3110	1009	4070	22.90	24.86	90.3	9
12	63	66.6	1.72	13.84	44.3	4.92	2.0	—	2829	2856	1387	4216	30.05	30.60	80.4	8
13	53	46.8	1.41	12.70	41.0	4.29	1.1	0.4	1944	1963	1161	3105	18.46	19.14	80.8	10
14	59	60.9	1.60	11.14	38.5	4.41	1.5	1.1	2301	2327	1093	3394	30.53	32.49	82.5	10
15	52	46.4	1.40	12.02	39.5	4.79	0.85	0.9	1988	2038	1015	3003	25.51	24.48	87.0	10
16	48	60.5	1.60	11.00	35.4	4.22	1.1	—	—	2463	1112	3575	15.04	16.38	88.9	12
17	54	39.6	1.33	10.90	36.2	4.29	1.8	0.7	—	2749	1128	3877	34.64	38.71	85.0	7
18	68	60.9	1.56	10.05	36.1	4.50	0.6	—	2437	2842	1250	3687	29.96	32.94	99.1	6
19	46	57.3	1.58	10.68	35.6	4.81	0.95	—	2458	2424	1104	3528	16.59	18.58	84.9	12
20	62	60.5	1.58	11.32	34.5	4.05	—	—	2306	2245	1018	3263	29.61	32.86	78.2	12
II. MALE PATIENTS																
21	56	81.8	1.95	13.00	40.2	4.24	—	—	3541	3543	1763	5216	49.60	52.80	75.5	14
22	61	40.9	1.42	11.00	37.1	4.07	1.7	1.7	2295	2386	1263	3558	29.52	27.26	91.7	14
23	67	52.3	1.58	12.35	41.0	4.24	0.5	0.8	2913	3004	1534	4447	48.66	48.54	71.4	10
24	60	68.2	1.80	12.97	43.4	4.60	1.7	1.3	3557	3725	2027	5584	56.93	59.25	79.6	7
25	61	65.5	1.85	13.45	45.0	5.15	1.4	—	3176	3450	2022	5190	37.79	36.84	75.0	8
26	38	71.4	1.83	15.10	48.3	5.41	1.9	—	2505	2615	1385	3890	24.17	25.27	80.0	7
27	67	58.6	1.69	11.00	37.5	4.08	0.8	—	2531	2623	1480	4011	27.13	27.26	79.5	12
28	41	57.7	1.62	12.78	41.7	4.95	0.7	0.4	3015	2929	1570	4499	37.70	40.16	87.3	10
29	43	82.7	2.09	13.45	43.3	4.78	1.3	—	2934	2238	1784	4622	29.04	30.34	79.9	10
30	39	69.5	1.85	7.19	26.1	3.64	0.7	0.7	3668	4517	1059	4727	29.21	33.80	89.9	7
31	20	82.7	2.00	13.34	41.5	4.31	1.25	—	3554	3622	1823	5377	35.59	28.94	89.1	10
32	44	56.8	1.51	11.67	41.9	4.27	1.1	—	—	3700	1118	4818	29.88	30.12	89.2	7
III. FEMALE CONTROLS																
1	30	50.9	1.50	13.05	39.5	4.17	0.2	—	2530	2436	1272	3808	23.79	29.36	79.0	9
2	63	61.8	1.65	14.02	43.4	4.72	—	—	2304	2085	1289	3374	22.30	29.16	72.5	17
3	43	68.2	1.66	13.34	41.6	4.54	—	—	2176	2291	1292	3470	26.76	34.95	90.6	17
4	64	75.0	1.79	11.67	36.0	4.26	—	—	2936	2950	1382	4318	23.56	27.68	84.8	6
5	56	74.1	1.86	13.45	41.8	4.41	—	—	2340	2557	1380	3720	17.95	21.26	75.6	16
IV. MALE CONTROLS																
6	62	63.2	1.66	14.60	44.4	4.90	0.25	0.9	2554	2443	1575	4018	24.12	25.43	83.6	13
7	35	69.5	1.85	16.50	50.5	5.68	0.2	—	2516	2583	1883	4399	23.41	27.33	78.4	16
8	34	90.0	2.06	15.00	46.5	5.47	0.1	2.0	3214	3227	2226	5440	33.00	36.24	75.0	7
9	65	60.5	1.63	15.00	46.6	4.82	—	—	2557	2529	1637	4166	33.74	37.62	77.3	14
10	58	84.1	1.99	13.71	40.8	4.61	0.45	—	3358	3637	1856	5214	34.15	37.25	86.2	14

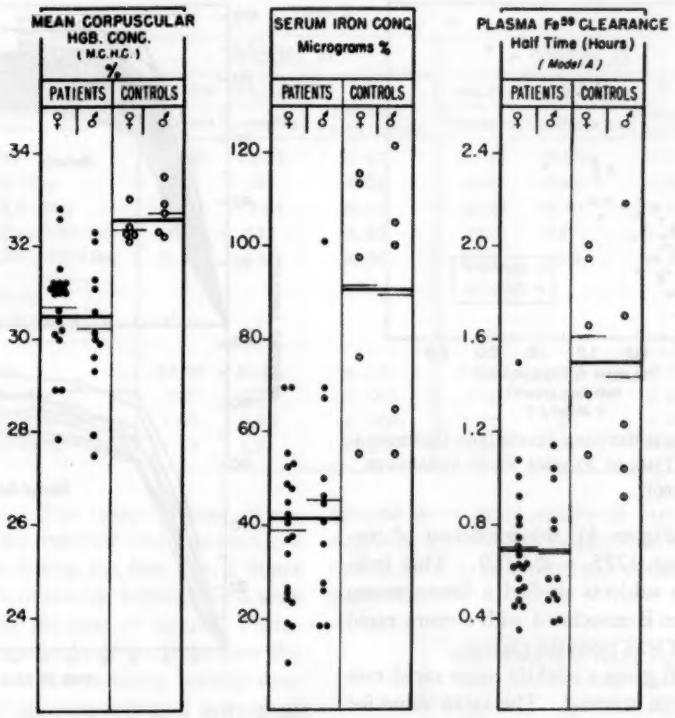

FIG. 2. VALUES OF M.C.H.C., SERUM IRON CONCENTRATION, AND HALF-TIME OF PLASMA FE-59 CLEARANCE IN RHEUMATOID ARTHRITIS PATIENTS AND CONTROLS

(90.8), and a lower average Mean Corpuscular Hemoglobin Concentration (30.5 per cent) than controls (32.6 per cent). It is of interest to note that 28 of the 32 patients studied had M.C.H.C. values below 32 per cent while none of the control subjects had a value below 32 per cent (Figure 2), indicating that the rheumatoid arthritis patients have a slightly hypochromic anemia. All patients showed an elevated erythrocyte sedimentation rate. No significant reticulocytosis was found in the patients. All of the patients studied had negative Coombs' tests.

B. Serum iron concentration

The mean serum iron concentration in the patients was significantly lower than that of the controls, the mean value for patients being 41.5 micrograms per cent and for the controls 90.5 micrograms per cent. Whereas a wide range of values was found in both groups (Figure 2), the standard error of the difference between the means was 8.80, giving a t value of 5.56. The probability that so large a difference between means was due to chance alone (p) is less than 0.001.

C. Radio-iron studies

1. Plasma radioiron removal rate. The period of time in hours required for the removal from the plasma of one-half of the transferrin-bound radioiron is referred to as the "half-time of plasma radioiron removal" (clearance). Using Model A, the mean half-time for the patients (0.70 hour) was definitely shorter than for the controls (1.53 hours). The distribution of these values is shown in Figure 2. The difference between these means is highly significant, the standard error of the difference being 0.137, the t value 6.05, and the p value less than 0.001. A definite linear relationship was found to exist between the serum iron concentration and the half-time of plasma radio-

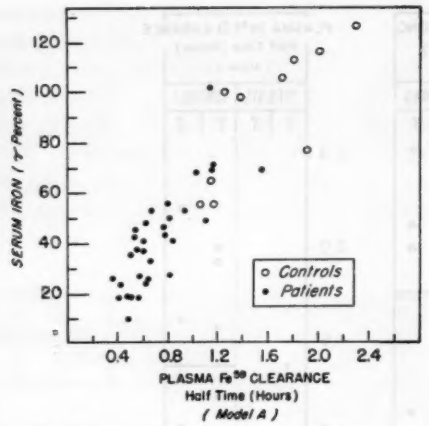

FIG. 3. RELATIONSHIP BETWEEN SERUM IRON CONCENTRATION AND HALF TIME OF PLASMA FE-59 CLEARANCE
$r = 0.77$, $p = < 0.001$.

iron clearance (Figure 3), the coefficient of correlation (r) being 0.775, $p < 0.001$. This indicates that in the subjects studied a lower serum iron concentration is associated with a more rapid rate of removal of iron from the plasma.

Using Model B gives a slightly more rapid rate of plasma radioiron removal. The mean value for half-time of plasma iron removal for patients was 0.65 hour and for controls 1.27 hours. The difference between these means was also significant, p being less than 0.001.

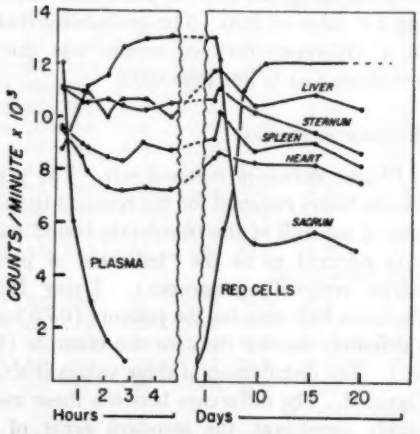

FIG. 4. EXTERNAL BODY MONITORING IN A RHEUMATOID ARTHRITIS PATIENT

Values for plasma and red cells were determined from measurement of blood samples. All other values were determined by external probe counting over indicated sites.

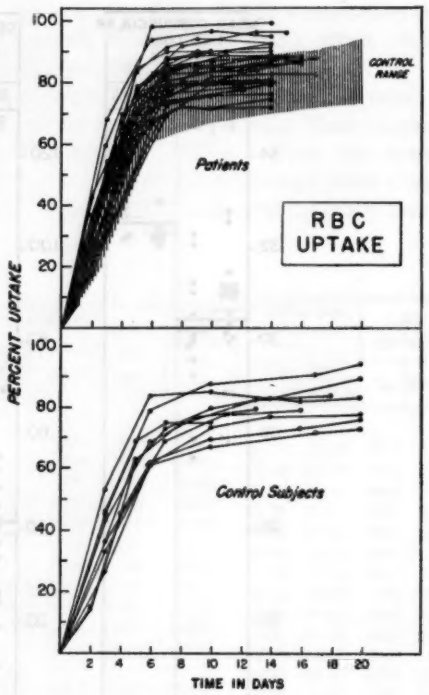

FIG. 5. RED CELL INCORPORATION OF INTRAVENOUSLY INJECTED RADIOIRON

2. External body monitoring. These studies were performed on 11 patients, 7 females and 4 males. All patients showed qualitatively the same

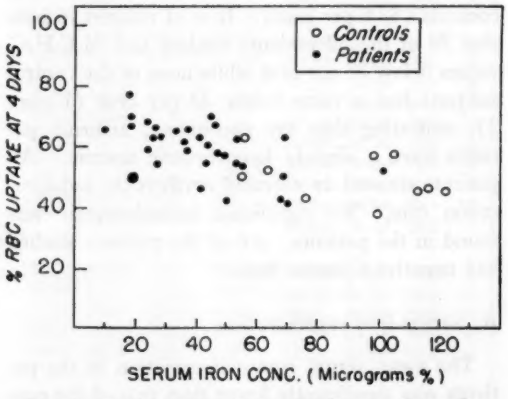

FIG. 6. RELATIONSHIP BETWEEN SERUM IRON CONCENTRATION AND THE RATE OF RED CELL UPTAKE OF RADIOIRON

The per cent uptake at four days is used as an index of the rate of incorporation of radioiron into red cells. $r = -0.63$, $p = < 0.001$.

Table II

	Model A			Model B		
	MEAN VALUE		P	MEAN VALUE		P
	Patients	Controls	Difference Pts. vs. Controls	Patients	Controls	Difference Pts. vs. Controls
PLASMA IRON TURNOVER RATE						
Mg. / Day	28.90	26.28	<.40	30.24	30.63	<.90
Mg. / Kg. / Day	.496	.383	<.02	.524	.448	<.20
Mg. / Sq.M. / Day	17.77	14.94	<.10	18.63	17.47	<.50
Mg. / 100ml. TBV / Day	.701	.631	<.20	.736	.741	>.90
Mg. / 100ml. RBC / Day	2.17	1.69	<.01	2.28	1.98	<.20
RBC IRON TURNOVER RATE						
Mg. / Day	24.44	21.15	<.20	25.59	24.67	<.70
Mg. / Kg. / Day	.417	.307	<.001	.445	.360	<.05
Mg. / 100ml. RBC / Day	1.85	1.36	<.001	1.95	1.60	<.05
Mg. / 100 mg. Hgb Fe / Day	1.79	1.21	<.001	1.89	1.42	<.01

pattern (Figure 4). The major change in activity occurred at the marrow site (sacrum), a rapid rise occurring during the first 4 to 5 hours after injection of the radioiron, followed by a slow continued rise during the next 24 hours. Thereafter, a rapid decline in activity occurred over the ensuing 3 to 6 days. The counting activity over the liver, spleen, and sternum reflected, in general, the changes observed in the blood measured over the heart, where a moderate fall in activity was observed during the first 24 hours followed by a moderate rise during the succeeding 3 to 7 days. This indicated that the major portion of radioiron removed from the plasma was deposited in the bone marrow, whence it was discharged into the blood in newly formed red cells. No evidence was found in these patients to suggest a diversion of plasma radioiron to other organs such as liver and spleen. The ferrokinetic pattern found in these patients was comparable to that found in normal subjects (25).

3. Red cell uptake of radioiron. The rate of radioiron incorporation into red cells is shown in Figure 5. Both patients and controls showed rapid uptake by red cells of 70 to 95 per cent of the injected radioiron in the 7 to 10 days following injection. The curves for the patients show a slightly more rapid initial slope of uptake, suggesting some relationship between serum iron concentration and the rate of red cell uptake. This relationship is shown in Figure 6, the percentage red cell uptake at 4 days serving as a measure of uptake rate. Subjects with lower serum iron concentrations showed more rapid uptake of injected radioiron. A similar relationship existed between the per cent uptake at 3, 5, or 6 days and the serum iron concentration.

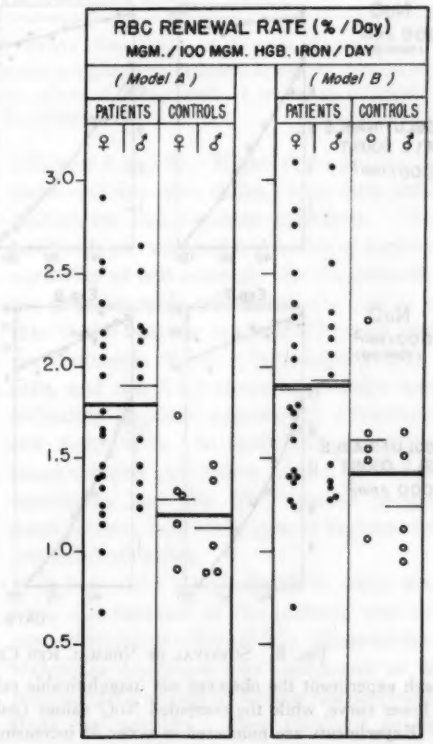

FIG. 7. VALUES FOR RED CELL RENEWAL RATE IN RHEUMATOID ARTHRITIS PATIENTS AND CONTROLS

4. Iron turnover rates (*Table II*). Using Model A, the mean value for the mgm. of plasma iron turned over per day was slightly but not significantly higher for patients than for controls. In both patients and controls, the mean values for males were definitely higher than for females, reflecting the larger body size of the males. When the iron turnover rates were corrected for body size the values for males and females were comparable. The mean plasma iron turnover rate corrected for body weight was significantly higher for patients than for controls. However, when the turnover rates were corrected for other parameters of body size such as surface area and total blood volume, the mean values for patients were not significantly higher than those for controls. There was no relationship between the serum iron concentration and the computed values for plasma iron turnover rate, the coefficient of correlation being 0.06, with a standard error of 0.15. Similarly, no correlation existed between serum iron concentration and plasma iron turnover rate corrected for body weight, nor between the half-time of plasma radioiron removal rate and plasma iron turnover rate.

The values for red cell iron turnover rates showed differences between patients and controls which were similar to those for plasma iron turnover rates. The rate of renewal of the circulating red cell mass can be computed by correcting the red cell iron turnover rate for red cell mass since one ml. of packed red cells contains approximately one mgm. of iron. The mean red cell renewal rate (mgm. per 100 ml. red cell mass per day) was significantly higher for patients than for controls. Because the patients had a lower average Mean Corpuscular Hemoglobin Concentration than controls, a more exact comparison of red cell iron renewal rates would be obtained on the basis of circulating hemoglobin iron. When red cell renewal rates were expressed as mgm. per 100 mgm. hemoglobin iron per day, the difference between patients

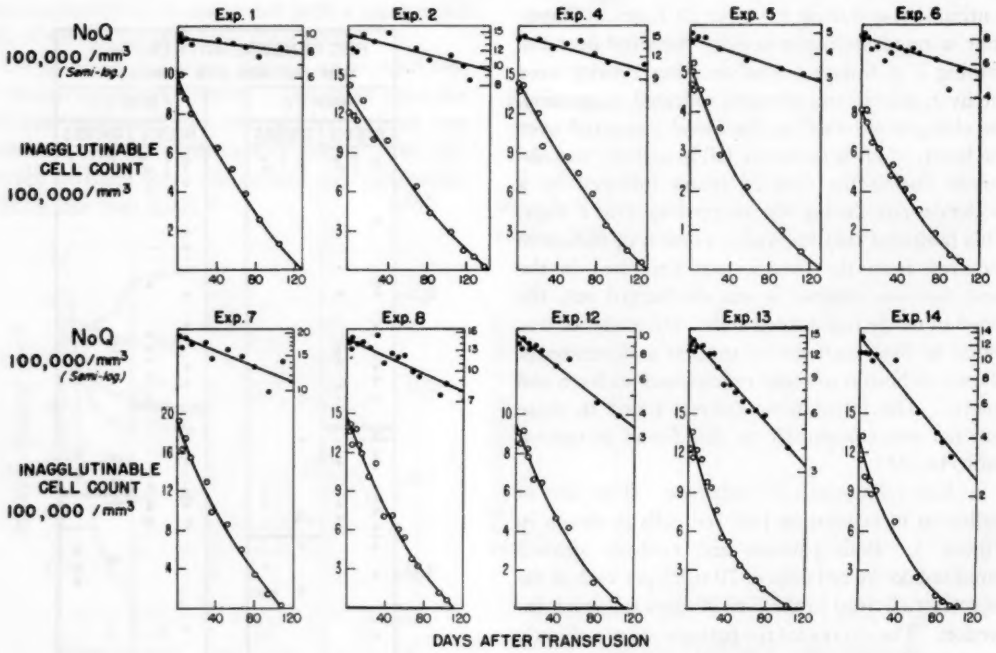

FIG. 8. SURVIVAL OF NORMAL RED CELLS IN RHEUMATOID ARTHRITIS PATIENTS

In each experiment the observed net inagglutinable cell counts (open circles) are plotted on Cartesian Coordinates in the lower curve, while the computed NoQ values (solid circles) are plotted on semi-log coordinates in the upper curve. Experiments are numbered in order of increasing abnormality. Experiments Nos. 3, 9, 10, and 11 are omitted and shown in Figure 9.

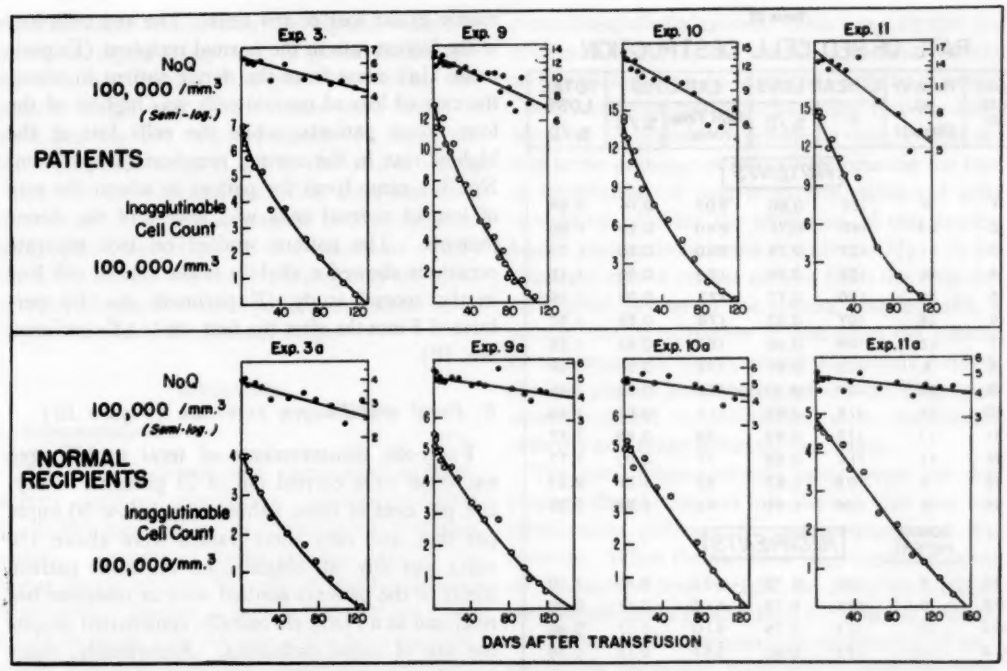

FIG. 9. SURVIVAL OF RHEUMATOID ARTHRITIS PATIENTS' RED CELLS IN NORMAL RECIPIENTS

The data on survival of patients' red cells in normal recipients are placed immediately below the data on survival of normal red cells in their respective donor patients. Thus, the patient of Experiment 11 served as the donor for the red cells whose survival in a normal recipient is shown in Experiment 11a.

and controls was greater, the mean value being significantly higher in patients.

Using Model B for computation of iron turnover rates gives slightly higher values than were obtained using Model A. This effect was more marked in controls than in arthritis patients, resulting in a decrease in the differences between means. The mean values obtained for plasma iron turnover rate using Model B show no significant differences between patients and controls, with or without corrections for body weight, surface area, blood volume, or red cell mass, p values exceeding 0.1 in all instances. However, the rate of red cell renewal or the red cell iron turnover rate expressed either as mgm. per 100 ml. of red cell mass or mgm. per 100 mgm. hemoglobin iron is still significantly greater for patients than for controls (Figure 7).

D. Erythrocyte survival studies

The survival of red cells from normal donors was studied in 13 rheumatoid arthritis patients (Figures 8 and 9). Eight of the patients were females and five were males. One male patient was studied on two separate occasions. These experiments are numbered in order of increasing abnormality of cell survival. In Experiment No. 1, the inagglutinable cell counts declined at an even rate to the baseline in about 120 days, indicating predominantly linear ("senescent") loss of red cells, and the NoQ curve is virtually horizontal, indicating minimal exponential ("random") red cell destruction. In successive experiments the inagglutinable cell count curves are increasingly curvilinear and the NoQ curves progressively more vertical, indicating greater degrees of random red cell destruction.

In four cases we were able to study simultaneously the survival of the patients' red cells in a normal recipient (Figure 9). None of the normal recipients developed any symptoms or signs of rheumatoid arthritis following transfusion of blood from the patients. One recipient developed a transient febrile episode, but otherwise the trans-

Table III

RATE OF RED CELL DESTRUCTION

SURV. EXP. NO.	PATIENT NO. (Table I)	LINEAR LOSS T	LINEAR LOSS %/D	EXP. LOSS Half Time (Days)	EXP. LOSS %/D	TOTAL LOSS %/D
		PATIENTS				
1	9	125	0.80	507	0.14	0.94
2	23	140	0.71	240	0.29	1.00
3	31	127	0.79	230	0.30	1.09
4	12	132	0.76	178	0.39	1.15
5	25	130	0.77	177	0.39	1.16
6	26	107	0.93	178	0.39	1.32
7	13	109	0.92	163	0.43	1.35
8	6	112	0.89	139	0.50	1.39
9	22	107	0.93	152	0.53	1.46
10	26	118	0.85	112	0.62	1.46
11	17	112	0.89	78	0.89	1.77
12	11	114	0.88	77	0.90	1.77
13	1	108	0.93	53	1.31	2.23
14	5	104	0.96	48	1.44	2.39
	DONOR PATIENT	RECIPIENTS				
3A	3	127	0.79	220	0.31	1.10
9A	9	137	0.73	410	0.17	0.90
10A	10	127	0.79	410	0.17	0.96
11A	11	153	0.65	537	0.13	0.78

fusions were uneventful. These experiments are indicated by the letter "a" and numbered to correspond with their respective donor patient. Thus, the patient studied in Experiment No. 3 served as the red cell donor for Experiment No. 3a. In all four normal recipients the inagglutinable cell count curves were virtually linear and the NoQ curves almost horizontal, indicating predominantly linear ("senescent") loss of the transfused patients red cells. In contrast, three of the donor patients showed an increased "random" loss of normal red cells (Experiments Nos. 9–11).

The rates of red cell destruction computed from these data are listed in Table III. The mean rate of red cell loss in the patients studied was 1.46 per cent per day, ranging from 0.94 per cent per day, a value within the control range, to 2.39 per cent per day, more than double the control rate. This increased rate of red cell loss was predominantly the result of an increased rate of exponential loss of red cells. The rates of linear loss showed only small differences. The mean rate of red cell loss in the normal recipients was 0.97 per cent per day, ranging from 0.78 per cent to 1.10 per cent per day. All the normal recipients showed predominantly linear loss of red cells. The red cells lost at the lowest rate in the normal recipient (Experiment 11a) came from the donor patient in whom the rate of loss of normal cells was highest of the four donor patients, while the cells lost at the highest rate in the normal recipient (Experiment No. 3a) came from the patient in whom the rate of loss of normal cells was lowest of the donor patients. The patient studied on two separate occasions showed a slightly lower rate of cell loss in the second study (Experiment No. 6) performed 5 months after the first study (Experiment No. 10).

E. Fecal urobilinogen excretion (Figure 10)

Forty-six measurements of fecal urobilinogen excretion were carried out in 21 patients. Fifty-five per cent of these values were below 50 mgm. per day, and only three values were above 150 mgm. per day, all obtained on the same patient. Many of the patients studied were at complete bed rest, and as a result chronically constipated despite the use of saline cathartics. Accordingly, many four-day stool specimens weighed less than 300 Gm. and yielded low values for fecal urobilinogen. In addition, any errors of measurement (*e.g.*, incomplete reduction or extraction in benzene) resulted in lower recoveries of urobilinogen. To

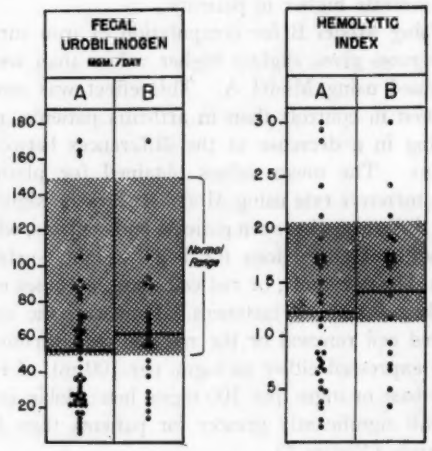

FIG. 10. FECAL UROBILINOGEN EXCRETION IN RHEUMATOID ARTHRITIS PATIENTS

All of the values obtained are shown in Column A. The highest value of repeat determinations in any given patient is shown in Column B.

minimize these effects only the highest value obtained on repeat samples in a given patient are shown in scattergram B, Figure 10. Using this method, 38 per cent of the values were below 50 mgm. per day and only one above 150 mgm. per day. The mean value is still in the normal range.

Rates of urobilinogen excretion were corrected for red cell mass and expressed as hemolytic indices. The mean of all the determinations (12.1) was in the low normal range. Using only the highest value obtained on each patient, the mean value (14.2) fell within the normal range.

DISCUSSION

A. Iron metabolism

This study indicates that rheumatoid arthritis patients utilize circulating transferrin-bound plasma iron for erythrocyte production in a normal manner. Seventy to ninety-five per cent of the plasma iron is utilized for red cell production and no significant amount of iron is diverted from the plasma to organ sites other than the bone marrow. In the patients studied, the plasma radioiron was removed more rapidly from the plasma and incorporated more rapidly into red cells than it was in the normal subjects. This difference may be explained on the basis that the radioiron was introduced into a smaller plasma iron pool in the patients, since the lower the serum iron concentration, the more rapid the rate of removal from the plasma and the rate of incorporation into red cells of the injected radioiron.

Previous studies of iron metabolism in patients with chronic inflammation, including patients with rheumatoid arthritis, have shown diminished red cell utilization (26–28), and increased tissue deposition of injected radioiron (29). These studies differed from the present one in that the injected radioiron was not bound to transferrin, and the amounts of carrier iron injected often exceeded the total iron-binding capacity of the plasma. More recent studies using trace quantities of radioiron in patients with chronic infection (30) and rheumatoid arthritis (31) have given results similar to ours. Thus, while patients with chronic inflammation show unimpaired red cell uptake of circulating plasma iron, they do show impaired uptake of relatively large quantities of unbound iron, the greatest proportion of which is deposited in the reticulo-endothelial tissues. This suggests that the release of iron from the reticulo-endothelial tissues to the plasma may be impaired in these patients. Confirmation of this hypothesis has recently been obtained in animal experiments which indicate that in the presence of sterile inflammation the rate of reutilization of iron from non-viable red cells is reduced, whereas the utilization of transferrin-bound plasma iron is not impaired (32). A diminished rate of release of iron from the reticulo-endothelial system to the plasma, coupled with a normal rate of migration of iron from the plasma to erythropoietic tissue, would adequately explain the low plasma iron concentration that is so consistently found in association with rheumatoid arthritis and other inflammatory states.

The total amount of iron turned over per day through the plasma and into the red cells was not significantly different for the patients and the controls. When the turnover rates were compared on the basis of body weight, the rates for patients were significantly greater than for controls. However, because the patients are chronically ill and are poorly nourished, body weight could be criticized as an unsatisfactory basis for comparison with normals. This was emphasized by the fact that the use of body surface area or total blood volume as a basis for comparison showed no significant difference between patients and controls. Thus, the mean rate of hemoglobin production was similar for patients and controls. Since the patients had a decreased circulating red cell mass and a normal rate of hemoglobin production, it follows that the rate of red cell renewal should have been increased. Computation of red cell renewal rates did give significantly higher rates for patients than for controls, the patients showing a mean renewal rate 1.5 times that of controls. Since the patients had fairly constant hemoglobin levels, the rates of red cell destruction were probably similar to the rates of cell renewal. This indicates that the patients have a higher rate of red cell destruction, or expressed in another way, a shorter red cell life span than the controls.

As described above, the turnover rates for plasma and red cell iron have been computed using the model proposed by Huff and his co-workers (10) (Model A). This model describes plasma radioiron clearance as an exponential function of time. However, careful inspection of our

data revealed a slight but definite curvilinearity, as has also been reported by Sharney, Schwartz, Wasserman, Port, and Leavitt (33). The mean curvature was found to be greater for patients ($c = 5.38 \times 10^{-5}$) than for controls ($c = 4.56 \times 10^{-5}$). For this reason Model B was developed and introduced. This model probably does not have great biological significance but it does allow an estimate to be made of the effect of this curvature on the observed differences. Accordingly, the turnover rates were recalculated using Model B in which plasma radioiron removal is assumed to be curvilinear. While the resulting differences between patients and controls were slightly decreased, these data confirmed the findings derived from Model A. Again, plasma iron turnover rates were not significantly different for patients and controls, while the red cell renewal rates were significantly greater in patients than controls.

These estimates of red cell production rates are based on data obtained during the morning hours only. A diurnal variation in plasma iron concentration (34, 35) and plasma iron turnover rate (36) has been demonstrated. It is possible that this diurnal variation is sufficiently different for patients and controls to alter the data presented. However, the rate of plasma iron removal was related to the plasma iron concentration, suggesting that a decrease in plasma iron concentration would be associated with an increased rate of removal of iron from the plasma. The rate of plasma iron turnover was not related to the plasma iron concentration suggesting that a decrease in plasma iron concentration would not be associated with any consistent change in plasma iron turnover rate. In addition to diurnal variation, errors in measurement of blood volume, serum iron levels, plasma radioiron clearance rates and perhaps unknown factors, may influence the computation of red cell renewal rates. As a result, the use of this method for detecting moderate alterations of red cell production in individual instances is often difficult. However, in the present study, patients and controls were studied under identical conditions and subject to similar errors. The number of subjects studied, the high level of significance of the difference between patient and control groups, and the confirmation of these data obtained with the use of Model B would indicate that the patients studied do show a moderate increase in red cell renewal rate when compared to controls.

B. Red cell survival

More than half of the rheumatoid arthritis patients studied showed increased rates of destruction of normal red cells. This increase in erythrocyte destruction was primarily the result of increased random loss of red cells. The potential life span of the red cells was not significantly shortened. When the cells from an arthritis patient were transfused into a normal recipient they showed a normal survival. This would indicate that the patients' red cells are not intrinsically defective, and that a defect extinsic to the red cell is responsible for the shortened survival in the patient.

If the patient's own red cells are destroyed in the same manner as the transfused normal cells, then patients showing increased rates of random red cell destruction should have a cell population containing a higher proportion of young red cells. When the younger red cell population is transfused into a normal recipient the rate of red cell loss should be less than normal. The data obtained from transfusion of patients' cells into normal recipients suggest that this was actually the case. The destruction rate of patients' cells was lower in the normal recipients when the destruction rate of normal cells was faster in the donor patient. A recent study by Alexander, Richmond, Roy, and Duthie (7) has also shown shortened survival of normal red cells in arthritis patients. They found in addition that red cells from an arthritis patient when transfused into another arthritis patient showed a cell survival which was better than that of normal red cells in that patient. These findings are consistent with the hypothesis that the arthritis patients have a cell population containing a greater proportion of young cells which show better survival than blood from a normal donor containing a normal cell population of all ages. These observations suggest that rheumatoid arthritis patients frequently show increased rates of destruction of their own circulating red cells. Similar findings of shortened red cell survival have been reported in anemias complicating cirrhosis, uremia (37, 38), and malignant diseases (39, 40). No immune mechanism could be

detected, and the cause of the shortened cell survival is not known.

Normal cells have been found to disappear in approximately linear fashion at a rate of 0.85 to 1.0 per cent per day in normal subjects. Some females who are apparently normal show slightly shortened red cell survival (41). This could account in part for the greater rates of red cell destruction found in the female patients in this series. However, half of our male patients showed increased destruction, while three-quarters of the females showed abnormal survival. Cells from a female patient were capable of normal survival when transfused into a normal recipient. Finally, a decrease in red cell survival regardless of cause would require an increase in red cell production to maintain normal hemoglobin levels.

When destruction rates estimated from survival data were compared with renewal rates estimated from radioiron data in each individual patient studied, a poor correlation was found, the coefficient of correlation being 0.3. Because of the small number of patients studied with both techniques, the 95 per cent confidence limits of this coefficient ranged from -0.2 to 0.7. The inadequacy of the sample size for such a correlation is emphasized by the fact that elimination of a single subject (Survival Exp. No. 2) results in a correlation coefficient of 0.5. Thus, these data do not establish the presence or absence of a positive correlation between these measurements. In addition, the study was not designed to test this relationship, since these two measurements were made at different times, separated by a 2 to 3-week interval. Moreover, these measurements were made over very different periods of time, the radioiron measurements being made over a 4 to 6-hour period, while the survival measurements were made over a 4-month period. Only if the rates of cell production and destruction were constant for the 5-month period of study, or if these two measurements were made simultaneously over similar periods of time, could such a correlation be expected. For these reasons, data obtained with each method in patients were compared with controls studied with the same method, under identical conditions. When this was done the data obtained from the survival studies are in agreement with the radioiron data, indicating a moderate increase in rates of cell destruction in the patients studied. The mean rate of cell destruction computed from survival studies was 1.55 times greater for patients than for controls and the mean rate of red cell renewal computed from radioiron data was 1.48 times greater for patients than for controls.

C. Fecal urobilinogen excretion

The rheumatoid arthritis patients were found to excrete normal to decreased quantities of fecal urobilinogen. Similar findings have been previously reported (8, 42). This indicates that normal amounts of hemoglobin are destroyed per day in the patients, which confirms the findings of the radioiron studies. It should be emphasized that the increased rates of cell renewal and destruction found with the radioiron and survival techniques do not necessarily indicate that an increased quantity of red cells are destroyed, since an increased percentage of cells destroyed together with a smaller red cell mass could result in a normal or even decreased quantity of hemoglobin breakdown. When urobilinogen excretion was related to red cell mass and expressed as hemolytic index, the mean value for the patient group is within the normal range. This does not agree with the above findings of increased rates of red cell renewal and destruction. However, chronic constipation in the bed-ridden arthritis patients could have resulted in falsely low values for fecal urobilinogen (43). Moreover, this method may not be sufficiently sensitive or accurate to detect the moderate increase in rates of hemoglobin destruction demonstrated by the other methods.

D. The mechanism of the anemia in arthritis patients

The mean values obtained in our patients indicate that the rate of cell destruction was increased while the quantity of red cell production was comparable to normal controls. Anemia results because erythropoiesis fails to increase in order to compensate for the increased hemolysis. While this describes the average arthritis patient, there were patients with normal red cell life span and diminished cell production. There were other patients showing shortened red cell life span associated with an increase in cell production, which, however, was inadequate to compensate for the increased rate of destruction. In all of these situ-

ations the bone marrow fails to respond adequately to the stimulus of anemia. Increased hemolysis is an additional factor in the production of anemia, however, since red cell production in normal marrow can increase 6 to 8 times (44), it is apparent that red cell production does not increase in the arthritic patient to a degree possible in the normal.

A mild but definite hypochromia of the red cells was present in the arthritis patients in the present study, as well as in other studies (1, 42, 45). Thus, the anemia of arthritis resembles morphologically the anemia of iron deficiency. Other similarities of these two anemias include a consistently decreased serum iron concentration, rapid removal of iron from the plasma, increase in serum copper, increase in free erythrocyte protoporphyrin, low serum bilirubin, and low fecal urobilinogen excretion (2). These similarities suggest that a diminished supply of iron for erythropoiesis may play a role in the impaired marrow function in these patients, despite adequate tissue iron stores (46). Failure of iron to be released from the reticulo-endothelial cells to the plasma at a normal rate could be responsible for hypoferremia and a decrease in the amount of iron available for red cell production. That this abnormality in iron metabolism does contribute to the anemia is indicated by the fact that a significant correlation was found to exist between the degree of hypoferremia and the severity of the anemia in our patients (Figure 11). This has also been found in other studies (1, 2). Moreover, if a diminished supply of iron to the plasma plays a role in the production of anemia, then correction of the hypoferremia for prolonged periods of time should improve the anemia. While studies of the effect of iron therapy have been variable, several investigators using large doses of intravenous iron given over prolonged periods have reported definite improvement in the anemia (42, 45, 47). Since the characteristics of the anemia of arthritis are similar to those of the anemia associated with chronic infection and inflammation, and to other secondary anemias (2), similar mechanisms may exist in these conditions.

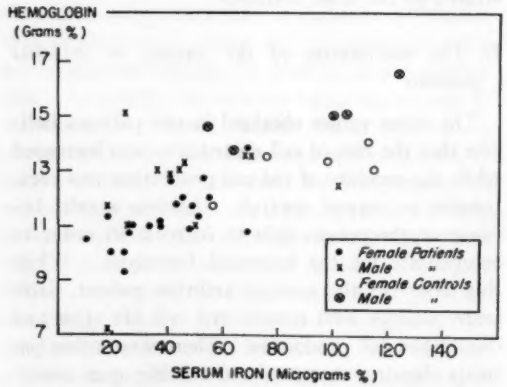

FIG. 11. RELATIONSHIP BETWEEN SERUM IRON CONCENTRATION AND HEMOGLOBIN LEVEL

$r = 0.68$, $p = < 0.001$.

CONCLUSIONS

Patients with rheumatoid arthritis show normal, unimpaired utilization of plasma iron for erythrocyte production. The amount of erythrocyte hemoglobin produced by these patients is approximately the same as in normal subjects. However, rates of red cell destruction and red cell renewal are abnormally increased. Increased red cell destruction results primarily from a greater random destruction of cells, the potential life span of the cells being virtually normal. The patients' erythrocytes proved capable of normal survival in a normal individual, indicating that the hemolytic mechanism in this disease is based on some factor associated with the red cell environment rather than on a defective quality of the red cell. Anemia reflects the failure of their bone marrow to respond adequately to the increased need for erythrocyte production. A defect in the release of iron from the R.-E. tissues to the plasma, resulting in hypoferremia and an inadequate supply of iron for red cell production, contributes to the impaired capacity of the bone marrow for red cell production.

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A STUDY OF THE QUANTITATIVE RELATIONSHIP BETWEEN ANTIDIURETIC HORMONE (VASOPRESSIN) AND THE RENAL TUBULAR REABSORPTION OF WATER[1]

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There is little available information regarding the quantitative relationship between the rate of secretion of antidiuretic hormone (ADH) and the rate of reabsorption of water by the renal tubules. From the work of Shannon (1) and Lauson (2) it has been suggested that in both dogs and adult humans the rate of secretion of ADH is equivalent to the intravenous administration of vasopressin at rates varying from less than 0.1 to more than 0.7 milliunit per hour per kilogram of body weight. Utilizing the endogenous creatinine U/P ratios as an index of the renal response, these authors both demonstrated a curvilinear relationship between the rate of infusion of vasopressin and the response of the kidney. The lowest rates of infusion of vasopressin studied were sufficient to produce a marked reduction in the rate of urine flow and increase in the endogenous creatinine U/P ratios, and there do not appear to be any data which define the quantitative relationship between ADH and the renal reabsorption of water in the range in which the concentration of the urine is generally less than that of the plasma. The current studies were designed to establish this relationship with the additional hope that its characteristics might provide some insight with respect to the mode of action of ADH.

PROCEDURE AND METHODS

Each experiment was designed to compare the rate of urine flow (V) and the clearance of free water (C_{H_2O}) (3) during maximal water diuresis with the urine flow and clearance of free water during the infusion of vasopressin, at one or more constant rates.

The subjects were all apparently healthy young adult men of ages twenty to thirty-five. No food or water was allowed for 10 to 12 hours prior to all but three of the experiments. In three studies (R.W., D.R., and E.D., 1/10/55), an attempt was made to increase (R.W. and E.D.) or decrease (D.R.) the rate of solute excretion. R.W. and E.D. (1/10/55) received 25 grams of sodium chloride per day added to their diets for two days prior to the study, and a portion thereof on the morning of the study upon arising. D.R. received only water *ad lib.* plus 200 grams of sucrose per day for two days prior to the study. No smoking was permitted for 8 hours prior to or during each experiment. All experiments were started between 7:30 and 8:30 A.M. and lasted from 6 to 11 hours, during which the subjects remained recumbent. Urine was collected through an indwelling Foley catheter with the aid of manual supra-pubic pressure. Venous blood was collected under oil through a Cournand needle which remained in an antecubital or forearm vein throughout each study. Approximately 0.25 ml. of heparin (1:1,000) was injected into the Cournand needle after each blood sample was obtained. No food or water was ingested during the experiments.

The basic design of the studies was as follows: In order to suppress the endogenous secretion of ADH as completely as possible, a positive balance of water of approximately one liter was produced in each subject, usually during the first hour, by the intravenous infusion of a solution of 3½ per cent dextrose, 3½ per cent invert sugar, or 5 per cent invert sugar, in water. This positive balance was subsequently maintained by adjusting the rate of infusion so that it approximated the rate of urine flow (infusion controlled by an Omega Machine Company "blood" pump which compresses the infusion tubing). In some experiments a maximal water diuresis was observed initially, followed by one or more constant infusions of vasopressin. In other experiments this order was reversed, and in a few studies an infusion of vasopressin was bracketed by periods of maximal water diuresis. In one subject (R.S.), one rate of infusion of vasopressin (0.5 milliunit

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per hour) was compared with a preceding maximal water diuresis; another rate of infusion (1.0 milliunit per hour) was compared with a subsequent maximal water diuresis. All infusions of vasopressin and all maximal water diureses were continued until an equilibrium appeared to have been achieved; *i.e.*, an approximately constant rate of urine flow for at least 2 to 3 collection periods of 10 minutes or more. Venous blood was obtained through the Cournand needle near the end of each such series of "equilibrium" periods, usually at the start of the last period. Studies were eliminated if emotional factors appeared to have influenced the results, but it is obviously impossible to know that they were not involved in those reported.

Two separate lots of Pitressin®[5] were used: the first (RH-303 M, studies 1 through 5) was several years old but was used only during a three-month period and was reassayed (pressor activity) by the Parke, Davis and Company laboratories during that period; the second lot (B 189750, studies 6 through 9) was generously supplied new by Parke, Davis and Company and was assayed by them before and after the experiments. The first lot apparently had deteriorated by about 50 per cent, and this fact has been incorporated in the rates of infusion reported. The pre- and post-experimental assays on the second lot indicated no significant decrease in pressor potency. The appropriate dilutions of vasopressin were all prepared from freshly opened ampoules in 5 per cent invert sugar and water immediately before infusion, and were all administered intravenously at a constant rate of approximately 1 ml. per minute controlled by a Bowman finger-type infusion pump. The constancy of this pump was studied on several occasions and showed a variation of less than 0.1 ml. per minute over a period of 8 or more hours. The rates of infusion were checked in each experiment by timing the infusions and by weighing the bottles of vasopressin before and after completion of the infusions.

Inulin was administered intravenously as a 2½ per cent solution in 5 per cent invert sugar and water at a constant rate of approximately 1 ml. per minute. Because of technical difficulties subsequent to the experiments, most of the analyses for inulin were unsatisfactory and clearances of inulin cannot be reported.

The osmolal concentrations of the serum and urine were measured on the day of the study with a Fiske Freezing Point Osmometer which was calibrated to read osmolality at the time of each set of determinations (4). A qualitative test for glucose in the urine was performed by the standard Benedict's method.

The osmolal clearance (C_{osm}) (3) and the clearance of free water (C_{H_2O}) (3) were calculated from the following formulas:

$$C_{osm} = \frac{[U]_{osm}V}{[P]_{osm}} \qquad C_{H_2O} = V - C_{osm}$$

where $[U]_{osm}$ = osmolal concentration of urine, $[P]_{osm}$ = osmolal concentration of serum, and V = urine flow in ml. per minute.

The change in free water clearance (ΔC_{H_2O}) associated with each rate of infusion of vasopressin was calculated by subtracting the average free water clearance observed at "equilibrium" during the infusion of vasopressin from the free water clearance obtained in the same experiment during maximal water diuresis ($C_{H_2O}^{max} - C_{H_2O} = \Delta C_{H_2O}$). The values for ΔC_{H_2O} have all been corrected to a standard surface area of 1.73 square meters ($\Delta C'_{H_2O}$), the surface area of subjects being estimated from their height and weight. The change in flow of urine (Δ flow) and the change in C_{osm} (ΔC_{osm}) have been calculated in the same manner as has ΔC_{H_2O}.

The method of analysis of variance was utilized to examine the adequacy of the calculated regression equations.

RESULTS

The rates of excretion of urine, the osmolal concentrations of the urine and serum, and the rate of excretion of solutes for *all* "equilibrium" periods are shown in Table I. The *average* values during "equilibrium" of V, $[U]_{osm}$, C_{osm}, C_{H_2O}, Δ flow, ΔC_{osm}, ΔC_{H_2O} and $\Delta C'_{H_2O}$ are presented in Table II. The urine flow and free water clearance during maximum water diuresis varied significantly among subjects. Similarly, if all subjects are considered, the urine flow and free water clearance during an infusion of vasopressin bore no absolute relationship to the rate of the infusion. Except for three subjects (R.W. 10/17/54, E.D. 1/10/55, and D.R. 2/23/55), all values of C_{osm} during maximal water diuresis (C°_{osm}) were between 2.3 and 4.0 ml. per minute. The exceptions (C°_{osm} = 7.0, 7.2, 1.9, and 1.7 ml. per minute) were produced intentionally as explained under "methods" in an effort to examine the effect of the rate of solute excretion on the quantitative behaviour of vasopressin. In twelve of the seventeen observations, ΔC_{osm} was no greater than 0.5 ml. per minute. There was no demonstrated glycosuria during the "equilibrium" periods of most studies; in a few, during the relatively higher rates of infusion of vasopressin, slight glycosuria was detected but never exceeded approximately 1 gram per cent. It is therefore unlikely that glucose was ever a major constituent of the urinary solutes.

The data in Table II show a considerable degree of biologic variation, despite which it can be seen that there was a graded curvilinear relationship between the rate of infusion of vasopressin and the renal response. The relationship

[5] Both lots were derived from an indeterminate mixture of hog and beef pituitaries and were supplied through the courtesy of Dr. A. C. Bratton, Jr., of Parke, Davis and Company.

TABLE I

The effects of various rates of infusion of vasopressin—Measured and derived data during all "equilibrium" collection periods

Subject Date Weight Height Est. s.a.	Rate of infusion of vasopressin	Time	Urine			Serum
			Flow	Solute concentration	Rate of solute excretion	Solute concentration
	mU./hour	*minutes*	*ml./min.*	*milliosmolal*	*micro-osmoles/min.*	*milliosmolal*
R. S.	0	156–168	14.2	48.0	682	
8/22/54	0	169–178	14.6	47.0	686	
75.80 Kg.	0	179–191	14.6	45.0	657	272
180 cm.						
1.93 sq. M.	0.5	248–263	11.5	55.0	632	
	0.5	264–278	11.7	57.0	667	
	0.5	279–292	11.6	63.0	731	271
	1.0	355–373	13.6	60.0	816	
	1.0	374–391	13.7	60.0	822	
	1.0	392–416	13.5	57.0	770	269
	0	464–484	16.4	46.0	754	
	0	485–507	17.6	44.0	774	265
	0	508–518	17.0	45.0	765	
D. D.	9.8	155–169	7.0	100.0	700	
8/26/54	9.8	170–183	7.1	97.0	689	
70.05 Kg.	9.8	184–199	7.0	99.0	693	275
190 cm.						
1.96 sq. M.	2.5	241–260	13.1	56.0	734	
	2.5	261–274	13.8	54.9	758	
	2.5	275–289	14.2	51.9	738	273.5
	1.4	409–422	15.0	43.0	645	
	1.4	423–437	16.6	48.0	798	270.5‡
	0	485–499	19.3	39.0	753	269
	0	500–508	19.4	37.0	718	
J. A.	9.3	186–206	1.7	318.0	540	
9/20/54	9.3	207–225	1.7	293.0	498	
92.20 Kg.	9.3	226–247	1.9	313.0	594	269
187 cm.						
2.18 sq. M.	4.7	384–404	2.9	144.0	417	
	4.7	405–426	2.5	205.0	513	
	4.7	427–449	3.8	141.0	536	269
	0	553–565	15.1	43.1	652	270
	0	566–575	14.0	43.0	602	
E. D.	7.2	113–137	6.6	190.0	1,254	
9/30/54	7.2	138–158	6.3	177.0	1,115	271
74.55 Kg.						
177 cm.	2.1	280–300	11.8	60.8	718	
1.90 sq. M.	2.1	301–317	11.8	60.8	718	
	2.1	318–333	12.1	66.1	800	270
	0	478–495	22.2	52.0	1,155	
	0	496–511	24.5	48.0	1,176	
	0	512–526	20.5	44.0	902	
	0	527–538	19.6	46.0	900	266
R. W.*	0	57–98	24.7	88.7	2,192	
10/17/54	0	99–110	23.7	80.1	1,900	
74.70 Kg.	0	111–125	22.9	72.9	1,670	276
181 cm.	0	126–136	23.7	62.8	1,490	
1.94 sq. M.						
	0.4	216–233	18.6	48.8	909	
	0.4	234–253	18.3	47.9	878	270
	0.4	254–273	18.7	48.0	899	

TABLE I—*Continued*

Subject Date Weight Height Est. s.a.	Rate of infusion of vasopressin	Time	Urine			Serum
			Flow	Solute concentration	Rate of solute excretion	Solute concentration
	mU./hour	*minutes*	*ml./min.*	*milliosmolal*	*micro-osmoles/min.*	*milliosmolal*
	1.9	383–402	9.9	85.5	847	
	1.9	403–422	8.5	102.0	867	
	1.9	423–439	10.9	87.8	958	266
W. M.	1.8	161–187	1.85	397.0	734	276‡
1/3/55	1.8	188–223	1.1	436.0	480	
76.65 Kg.						
190 cm.	0	373–395	12.1	78.9	955	
2.06 sq. M.	0	396–415	10.9	70.0	763	268
	1.8	493–507	5.3	167.0	884	
	1.8	508–523	4.9	175.0	856	
	1.8	524–540	4.9	171.0	838	265
E. D.*	15.5	130–152	4.4	501.0	2,205	
1/10/55	15.5	153–172	4.2	542.0	2,278	
75.90 Kg.	15.5	173–192	4.0	565.0	2,258	274
177 cm.						
1.92 sq. M.	0	360–376	28.8	72.9	2,102	
	0	377–399	28.7	67.9	1,951	
	0	400–417	28.8	64.0	1,844	
	0	418–427	29.8	59.9	1,786	268
D. R.†	0	149–168	9.5	53.0	504	
2/23/55	0	169–189	11.2	51.9	582	
73.97 Kg.	0	190–207	10.0	52.7	527	278
182 cm.						
1.94 sq. M.	1.7	328–349	3.4	112.0	382	
	1.7	350–369	3.3	117.0	385	
	1.7	370–389	3.7	109.0	402	270
	0	461–479	9.1	55.1	502	
	0	480–499	8.3	53.7	446	
	0	500–519	8.5	52.0	442	
	0	520–530	9.6	50.7	487	271
T. C.	10.7	305–324	1.2	475.0	570	
5/6/55	10.7	325–344	0.9	584.0	526	
82.65 Kg.	10.7	345–364	0.9	600.0	540	271
188 cm.						
2.1 sq. M.	0	497–511	13.0	55.0	715	275
	0	512–526	12.9	51.8	669	
	0	527–540	13.2	51.0	674	

* High sodium chloride intake prior to study (see text).
† Low solute intake prior to study (see text).
‡ An interpolated value.

may be expressed by at least three simple regression equations, the renal response being expressed as $\Delta C'_{H_2O}$ since it appears reasonable to assume that the change in free water clearance represents the increase in the reabsorption of water by the renal tubules which results from each particular infusion of vasopressin (see Discussion). If ΔC_{osm} were zero, ΔC_{H_2O} would of course equal Δ flow. The equations (1, 2, and 3) and the results of analysis of variance are shown in Table III-A. All three equations are highly significant in a statistical sense, but at such extremely high values of "F," statistical considerations alone cannot determine which expression most accurately defines the relationship. Furthermore, the derivation of these particular equations is in no sense intended to imply that they represent the only expressions which will describe the data, since it is certain that many other more complex equations involving two or more constants would do so as well.

In order to minimize any effect which a change

TABLE II

The effects of various rates of infusion of vasopressin—Average "equilibrium" values

Subject Date	Rate of infusion of vasopressin	Urine							
		Flow	Solute concentration	C_{osm}	C_{H_2O}	Δ Flow	ΔC_{osm}	ΔC_{H_2O}	$\Delta C'_{H_2O}$
	mU./hr.	*ml./min.*	*milliosmolal*	*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*	*ml./min.*
R. S.	0	14.5	47	2.5	12.0				
8/22/54	0.5	11.6	58	2.5	9.1	2.9	0	2.9	2.6
	0	17.0	45	2.9	14.1				
	1.0	13.6	59	3.0	10.6	3.4	−0.1	3.5	3.1
D. D.	0	19.4	38	2.8	16.6				
8/26/54	1.4	15.8	45	2.7	13.1	3.6	0.1	3.5	3.1
	2.5	13.7	54	2.7	11.0	5.7	0.1	5.6	4.9
	9.8	7.0	99	2.5	4.5	12.4	0.3	12.1	10.7
J. A.	0	14.7	43	2.3	12.4				
9/20/54	4.7	3.1	163	1.8	1.3	11.6	0.5	11.1	8.8
	9.3	1.8	309	2.0	−0.2	12.9	0.3	12.6	10.0
E. D.	0	21.9	48	3.9	18.0				
9/30/54	2.1	11.9	62	2.8	9.1	10.0	1.1	8.9	8.1
	7.2	6.5	184	4.4	2.1	15.4	−0.5	15.9	14.5
R. W.*	0	24.1	81	7.0	17.1				
10/17/54	0.4	18.5	48	3.3	15.2	5.6	3.7	1.9	1.7
	1.9	9.7	92	3.3	6.4	14.4	3.7	10.7	9.5
W. M.	0	11.5	75	3.2	8.3				
1/3/55	1.8	1.4	419	2.1	−0.7	10.1	1.1	9.0	7.6
	0	11.5	75	3.2	8.3				
	1.8	5.0	171	3.2	1.8	6.5	0	6.5	5.5
E. D.*	0	28.9	67	7.2	21.7				
1/10/55	15.5	4.2	534	8.2	−4.0	24.7	−1.0	25.7	23.4
D. R.†	0	10.2	53	1.9	8.3				
2/23/55	1.7	3.4	113	1.4	2.0	6.8	0.5	6.3	5.6
	0	8.7	53	1.7	7.0				
	1.7	3.4	113	1.4	2.0	5.3	0.3	5.0	4.5
T. C.	0	13.0	53	2.5	10.5				
5/6/55	10.7	1.0	553	2.0	−1.0	12.0	0.5	11.5	9.5

* High NaCl diet (see text).
† Low solute diet (see text).

TABLE III

Regression equations and the results of analysis of variance

Equation	Analysis of variance	
	"F"*	Variation in data "removed" by the equation (% of total)
(A) All data		
(1) $\Delta C'_{H_2O} = 3.89 + 9.39 \log$ (vasopressin rate)	32.0	68.1
(2) $\log \Delta C'_{H_2O} = 0.570 + 0.564 \log$ (vasopressin rate)	63.6	80.9
(3) $1/\Delta C'_{H_2O} = 0.071 + 0.196$ (1/vasopressin rate)	108	87.8
(B) Data associated with ΔC_{osm} of 0.5 ml. or less		
(4) $\Delta C'_{H_2O} = 3.44 + 7.58 \log$ (vasopressin rate)	37.9	79.1
(5) $\log \Delta C'_{H_2O} = 0.542 + 0.519 \log$ (vasopressin rate)	70.6	87.6
(6) $1/\Delta C'_{H_2O} = 0.098 + 0.173$ (1/vasopressin rate)	42.1	80.7

* The probability of "F" occurring by chance is less than 0.001 for each of the equations.

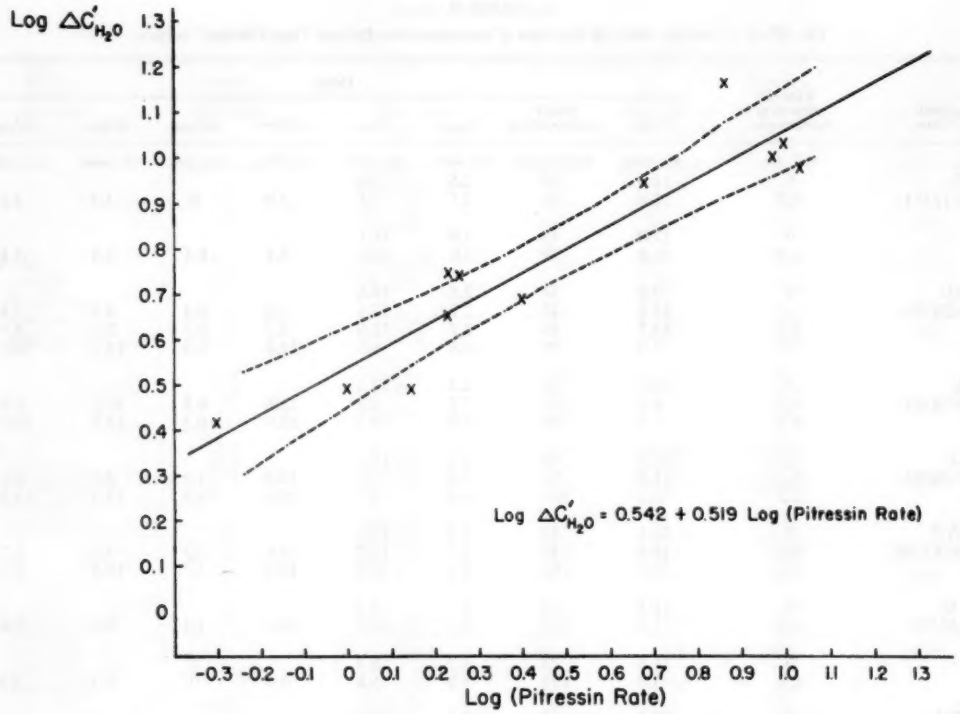

FIG. 1. THE RELATIONSHIP BETWEEN $\Delta C'_{H_2O}$ (ML. PER MINUTE) AND THE RATE OF INFUSION OF VASOPRESSIN (MILLIUNITS PER HOUR) EXPRESSED AS A LOG-LOG TYPE OF FUNCTION

The dotted lines represent the 95 per cent confidence limits for the *mean* values of log $\Delta C'_{H_2O}$ (not for individual values).

in the rate of excretion of solutes might have on $\Delta C'_{H_2O}$, the data have been reconsidered after eliminating the five studies associated with a ΔC_{osm} of more than 0.5 ml. per minute. This also eliminates four of the five studies in which C°_{osm} was greater than 3.5 ml. per minute. The resulting regression equations (4, 5, and 6) are shown in Table III-B. Equations 5 and 6 are also illustrated in Figures 1 and 2. Again, statistical analysis revealed that the three types of equation are very well approximated by the data. It will also be noted that, if the one point representing a rate of infusion of vasopressin of 0.5 milliunit per hour were omitted, the data would describe a regression equation of the type shown in Figure 2 (Equation 6) with considerably less variation about the line.

That these three types of mathematical expression should all provide such excellent descriptions of the data is not surprising because, as shown in Figure 3, these three equations approximate each other quite closely throughout most of the dose range involved in their derivation (0.5 to 10.7 milliunits per hour). Obviously, it would be extremely difficult to differentiate between them in this range on the basis of data as variable as that provided by most biological studies.

It should be emphasized that in all but one study (E.D. 1/10/55) the rates of infusion of vasopressin were less than that required to produce a significantly hypertonic urine and the relationships defined may not be applicable at higher rates of administration of vasopressin.

The method of analysis of variance was utilized to examine the influence of the rate of excretion of solutes on the renal response to vasopressin. The variable considered was the value of C_{osm}

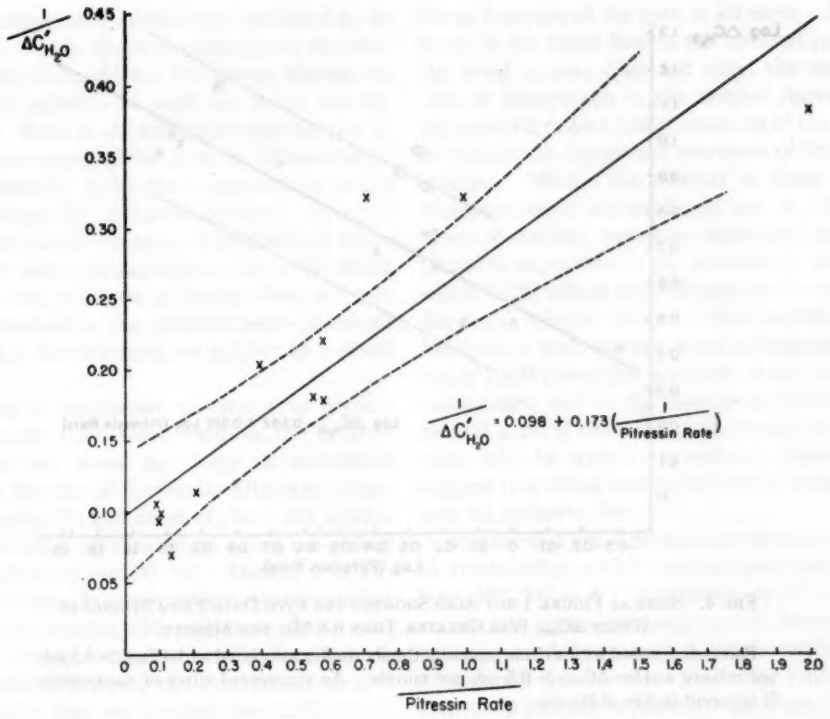

FIG. 2. THE RELATIONSHIP BETWEEN $\Delta C'_{H_2O}$ (ML. PER MINUTE) AND THE RATE OF INFUSION OF VASOPRESSIN (MILLIUNITS PER HOUR) EXPRESSED AS A RECIPROCAL TYPE OF FUNCTION
The dotted lines represent the 95 per cent confidence limits for the *mean* values of $1/\Delta C'_{H_2O}$.

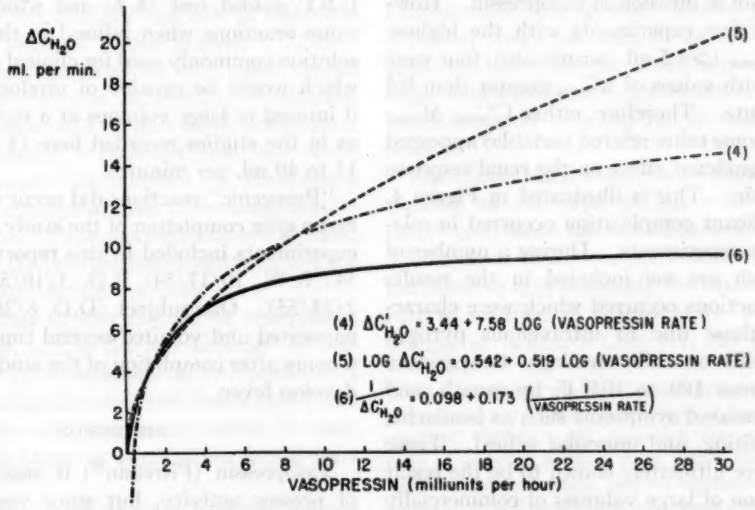

FIG. 3. EQUATIONS 4, 5, AND 6 EXTRAPOLATED IN BOTH DIRECTIONS FROM THE RANGE OF THE ACTUAL DATA

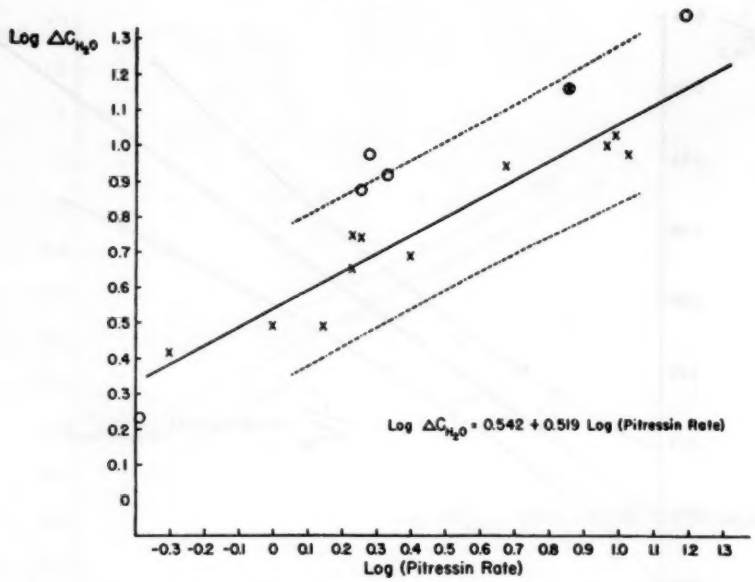

FIG. 4. SAME AS FIGURE 1 BUT ALSO SHOWING THE FIVE DATA FROM STUDIES IN WHICH ΔC_{osm} WAS GREATER THAN 0.5 ML. PER MINUTE

Points designated with a circle represent the six studies associated with $C^{\circ}_{osm} > 3.5$ ml. per minute and/or $\Delta C_{osm} > 0.5$ ml. per minute. An augmented effect of vasopressin is apparent in five of the six.

during maximal water diuresis (C°_{osm}), and it appeared that with increasing values of C°_{osm} there was a slight increase in the $\Delta C'_{H_2O}$ for any particular rate of infusion of vasopressin. However, of the five experiments with the highest values of C°_{osm} (>3.5 ml. per minute), four were associated with values of ΔC_{osm} greater than 0.5 ml. per minute. Therefore, either C°_{osm}, ΔC_{osm} or both (or some other related variable) appeared to have a significant effect on the renal response to vasopressin. This is illustrated in Figure 4.

One significant complication occurred in relation to these experiments. During a number of studies which are *not* included in the results reported, reactions occurred which were characteristic of those due to intravenous pyrogen (shaking chills or chilly feelings, temperature elevations from 100 to 103° F. by mouth, and variable associated symptoms such as headache, nausea, vomiting, and muscular aches). These reactions were ultimately shown to be the result of the infusion of large volumes of commercially produced "pyrogen-free" parenteral fluid. This fluid was always found to be bacteriologically sterile and it is presumed that it contained minute amounts of pyrogenic material, which were sufficiently small to escape detection by the standard U.S.P. rabbit test (5, 6) and which would not cause reactions when infused in the volumes of solution commonly used for clinical purposes, but which would be capable of producing reactions if infused in large volumes at a rapid rate (5, 6) as in the studies recorded here (5 to 9 liters at 15 to 40 ml. per minute).

"Pyrogenic" reactions did occur during the 12 hours *after* completion of the study in four of the experiments included in this report (R.S. 8/22/54; R.W. 10/17/54; E.D. 1/10/55; and D.R. 2/23/55). One subject (D.D. 8/26/54) became nauseated and vomited several times during the 6 hours after completion of the study but did not develop fever.

DISCUSSION

Vasopressin (Pitressin®) is assayed in terms of pressor activity, but since vasopressin and antidiuretic hormone are probably identical (7), the pressor and antidiuretic activities should

bear a consistent quantitative relationship to each other. It is therefore reasonable to combine results obtained from two lots of Pitressin®, the pressor activity of each lot being known. Obviously, there is no particular significance in the absolute values of the rates of infusion since these necessarily have been assigned in terms of the biologically assayed "pressor" activity. Also, these calculated rates of infusion of vasopressin are only approximations, since the assay method is not accurate to better than ±7 per cent (8), and since the measurements involved in preparing the dilutions are subject to a small error.

Infusions of vasopressin at rates even considerably greater than those used in the current studies do not cause any large or consistent changes in the rate of glomerular filtration (clearance of inulin) (9) and cause slight if any alteration in total effective renal plasma flow (clearance of para-amino-hippurate) (9). Hence, it is unlikely that hemodynamic effects were involved in the current results, although such effects cannot be as clearly eliminated in the four experiments in which a pyrogenic reaction developed subsequent to (but not during) the study.

There are three fundamental assumptions implicit in the experimental design of these studies, the validity of which cannot be known: 1) That there was no significant secretion of endogenous ADH during "equilibrium" periods of what has been called "maximal water diuresis"; 2) that the changes in C_{H_2O} observed with the infusions of vasopressin were due entirely to the administered vasopressin; and 3) that when vasopressin is being infused at a constant rate, and when C_{H_2O} is constant, the amount of vasopressin acting on the appropriate site in the renal tubules is also constant. These assumptions are, at least, supported by the approximate constancy of C_{H_2O} during the "equilibrium" periods.

If the volume of distribution of vasopressin does not alter, and if the *rate constant*[6] of biological decay of vasopressin is independent of concentration (as Ginsburg and Heller (10) found it to be in the rat), the concentration of vasopressin in the blood at equilibrium would be a linear function of the rate of infusion. Furthermore, if the blood flow to the affected portion of the renal tubules does not alter, the concentration of vasopressin in the critical region would be expected to be a linear function of the concentration in the blood and therefore of the rate of infusion. Within the context of these and the aforementioned assumptions, one of the three types of derived regression equations may be a plausible expression of the physiologic process by which ADH effects the abstraction of water from the renal tubular urine. Unfortunately, establishment of such mathematical relationships cannot of itself prove the existence of any particular mechanism, and in the discussion which follows several general concepts with respect to mechanism will be examined without intending to suggest that those mentioned in any sense represent an inclusive list.

Equations 1 and 4 express an exponential type of relationship which can be presented in the form $10^{\Delta C'_{H_2O}} = A\ (\text{Vasopressin rate})^B$. This, of course, is a commonly used type of dose-response equation, and if appropriate for the current data, it does not provide any particular insight with respect to possible mechanisms. Since this form of mathematical expression predicts unlimited values of $\Delta C'_{H_2O}$ at ever increasing rates of infusion of vasopressin, it obviously cannot be applicable much above the range actually studied. Furthermore, this type of equation predicts theoretically negative values for $\Delta C'_{H_2O}$ (which approach minus infinity as a limit) as the rate of infusion of vasopressin approaches zero as a limit, which is almost certainly not in conformity with reality.[7]

Equations 2 and 5 express an exponential relationship of the type $\Delta C'_{H_2O} = A\ (\text{vasopressin rate})^B$ and, since the exponent is approximately 0.5, imply that the reabsorption of water is proportional to approximately the square root of the rate of infusion of vasopressin. This is reminiscent of Schütz's Law (11, 12) which states

[6] The *rate constant* is the *proportion* of the total quantity of ADH present in its volume of distribution which leaves that compartment by any route in unit time.

[7] It is implicit in the experimental design of these studies that a rate of infusion of vasopressin of zero evokes a zero response ($\Delta C'_{H_2O} = 0$). Although the semi-logarithmic form of equation appears valid within the range of the actual experimental data, such an expression cannot define accurately the theoretical dose-response relationship in the range where the dose is approaching zero and when $\Delta C'_{H_2O}$ also would be expected to be approaching zero.

that the rate of certain enzymatic reactions is a function of the square root of the enzyme concentration,[8] thereby suggesting the possibility that ADH may act as an enzyme or apo-enzyme for a chemical reaction effecting the reabsorption of water. Since this type of equation also predicts unlimited values of $\Delta C'_{H_2O}$ at ever increasing doses of vasopressin, it (like equations 1 and 4) cannot be applicable much above the range studied.

Relationships of the type represented by equations 3 and 6 can be derived from several hypothetical modes of action of ADH. One such derivation[9] has been suggested by Dr. Hans Ussing (13) who proposes that each molecule of ADH is potentially able to combine reversibly in some way with a "site" in the renal tubular cell or on its membrane, thereby creating a "pore" through which water can flow at a constant rate. The actual mechanism causing the flow of water is immaterial; the term "pore" merely indicates the rate-limiting element of the process. It is further assumed that the total number of potential "pores" is constant, that all potential "pore" sites have the same affinity for ADH, and that the rate of movement of water is proportional to the number of "pores." From these assumptions it is possible to derive the following equation:[9]

$$\frac{1}{\Delta W} = \frac{1}{M_0 a} + \frac{k}{M_0 a}\left(\frac{1}{H}\right)$$

where

ΔW = rate of reabsorption of water resulting from ADH.
H = number of available ADH molecules.
k = the dissociation constant for the combination of vasopressin with the so-called "pore" sites.
M_0 = total number of potential "pores" (constant).
a = constant.

Assuming that ΔW represents $\Delta C'_{H_2O}$ and that H is proportional to the rate of infusion of vasopressin, this is comparable to equations 3 and 6.

The conceptual approach suggested by Ussing would apply to any mechanism which satisfied the same general assumptions; *i.e.*, the reversible combination of ADH with some "receptor" in or on the renal tubular cells, a limited number of receptors, and a linear relationship between the number of ADH-receptor combinations and the rate of reabsorption of water. Furthermore, it is apparent that these assumptions and the reciprocal type of equation derived from them are essentially analogous to the Lineweaver-Burk expression of the Michaelis-Menten theory of enzyme kinetics. The possible usefulness of this approach to the physiologic interpretation of hormone dose-response relationships has also been recently suggested by Stetten (14).

Another concept from which it is possible to derive a relationship of the type expressed in equations 3 and 6 is that the effect of ADH is, in some unknown way, to raise the osmotically effective concentration in the renal tubular cells (thus promoting the osmotic reabsorption of water), and that each molecule of ADH results in a constant increment of that effective concentration up to a maximum.[10]

It will be noted that equations of the type just discussed, unlike equations 1, 2, 4, and 5, predict that at increasing rates of infusion of vasopressin, $\Delta C'_{H_2O}$ will approach a maximum (M_0a) asymptotically. It might therefore be implied that the process effected by ADH is limited by a maximum *rate*, an implication which is consistent with the current hypothesis (3, 15, 16) in which the "facultative" reabsorption of water by the renal tubules is considered to be a dual process involving: 1) $T^d_{H_2O}$, the rate of reabsorption of water in the distal tubule, under the influence of ADH but limited by the rate of reabsorption of solutes since its maximum is that which will produce a concentration isosmotic with the glomerular filtrate; and 2) $T^c_{H_2O}$, the rate of reabsorption of solute-free water at some site distal to that of $T^d_{H_2O}$, presumably not influenced by ADH, approximately constant and maximal ($Tm^c_{H_2O}$) at relatively high rates of solute excretion or whenever the final urine formed is hypotonic to plasma. If such a dual mechanism were in fact operative, the measured values of ΔC_{H_2O} in the current studies could be represented as follows: $\Delta C_{H_2O} = \Delta T^d_{H_2O} + \Delta T^c_{H_2O}$. Since the

[8] There appears to be considerable uncertainty with respect to the validity of this "law" for any enzymatic reaction.

[9] See Appendix A.

[10] See Appendix B.

final urine was almost always hypotonic to the plasma, $T^c_{H_2O}$ should equal $Tm^e_{H_2O}$, and as the latter is presumably constant, it could be assumed that, within the range studied, $\Delta C_{H_2O} = \Delta T^d_{H_2O}$. For this reason, it might be appropriate to consider the derived regression equation as a description of the relationship between the rate of infusion of vasopressin and $\Delta T^d_{H_2O}$. In this regard, it is interesting that the maximum values of the dependent variable predicted by equations 3 and 6 (14.1 and 10.2 ml. per minute,[11] respectively) are of the same order of magnitude as that anticipated for $\Delta T^d_{H_2O}$ from calculations based on the dual mechanism hypothesis[12] (15.8 ml. per minute[11]). Thus, although there is no particular basis for assuming that the relationship between ADH and $\Delta T^d_{H_2O}$ must be described by a single continuous regression equation, it appears that the reciprocal form (equations 3 and 6) is the only one of the three simple types discussed here which might reasonably be applicable much above the range of the current data.

The renal response to vasopressin or to an extract of the posterior pituitary gland has been examined in many animal studies (1, 2, 17–22). Most of these cannot be readily compared with the present experiments because of differences in technique and because the dose-response relationship often is uncertain. Nonetheless, at least two studies do allow the derivation of a quantitative relationship between several sub-maximal doses of injected material and the renal response. Ginsburg and Heller (22) examined the antidiuretic action of individual doses of vasopressin injected intravenously into unanesthetized rats. Table IV is based on Figure 1 of their report. Change in urine flow has been calculated from the flows shown before, and for varying periods after, injection of vasopressin. Maximum change in flow, Δ flow$_{max}$, is based on the lowest urine flow during any single period following the injection. Δ flow$_{20}$ and Δ flow$_{30}$ are based on averages obtained by including the urine flows for 20 and 30 minutes, respectively, after the injection of vasopressin. As the authors state, a plot of the logarithm of the dose against the response is well described by a straight regression line. As shown in Figure 5, the data (using Δ flow$_{30}$) are also well defined by either a log-log or reciprocal relationship. The regression coefficients are quite similar to those reported here: 0.601 for the log-log (compared to 0.564 and 0.519 in equations 2 and 5); 0.215 for the reciprocal relationship (compared to 0.196 and 0.173 in equations 3 and 6). Jeffers, Livezey, and Austin (19) studied the dose-response relationship of vasopressin injected intravenously (as individual doses) into water-loaded rats receiving alcohol, the response being defined as the difference between urine flow for 20 minutes after and 20 minutes before injection. Again, the results are well expressed by either a semi-logarithmic or a log-log type of regression equation, and somewhat less well by one involving reciprocals. The studies of Lauson (2) on a normal man and those of Shannon (1) on dogs with diabetes insipidus have been mentioned in the introduction. Both of these investigators utilized a technique which was similar to the one employed in the current experiment but the range studied generally was higher than in the current studies.

TABLE IV

Renal response of rats to vasopressin (from Ginsburg and Heller [22] Figure 1)

Dose of vasopressin	Δ flow$_{max}$*	Δ flow$_{20}$†	Δ flow$_{30}$‡
(milliunits)	*ml./10 min.*	*ml./10 min.*	*ml./10 min.*
0.1	0.6	0.4	0.4
0.2	1.0	0.85	0.6
0.4	1.4	1.3	0.9
0.6	1.8	1.65	1.3
0.8	1.8	1.75	1.3

* Δ flow$_{max}$ = Difference between pre-injection flow and minimum flow during any ten-minute period following injection.

† Δ flow$_{20}$ = Difference between pre-injection flow and the average flow during first twenty minutes following injection.

‡ Δ flow$_{30}$ = Same as Δ flow$_{20}$ except post-injection flow averaged for thirty minutes.

SUMMARY AND CONCLUSIONS

An attempt has been made to define the quantitative relationship between antidiuretic hor-

[11] All figures corrected to body surface area of 1.73 square meters.

[12] From Zak, Brun, and Smith (16) $Tm^e_{H_2O}$ in water-loaded adults = 4.1 ml. per minute. The average maximum C_{H_2O} in the current studies = 11.7 ml. per minute. The theoretically maximum $\Delta T^d_{H_2O}$ is the sum of $Tm^e_{H_2O}$ and the maximum C_{H_2O} = 4.1 + 11.7 = 15.8 ml. per minute.

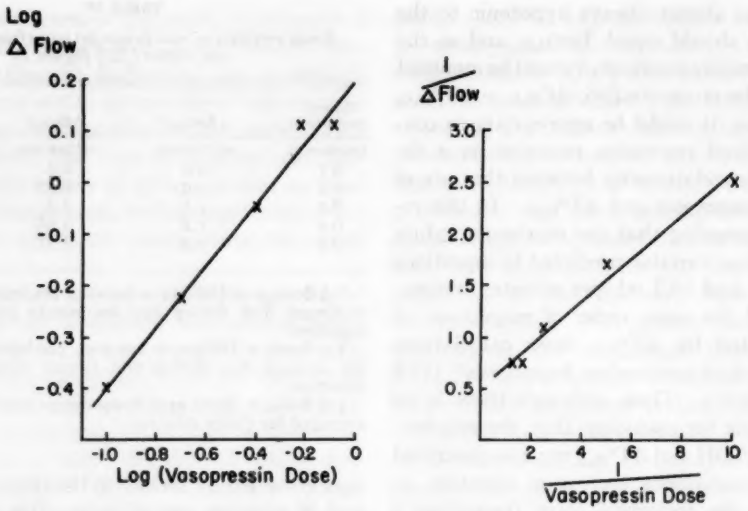

FIG. 5. THE RELATIONSHIP BETWEEN Δ FLOW (ML. PER 10 MINUTES) AND THE DOSE OF VASOPRESSIN (MILLIUNITS) IN RATS, BASED ON DATA OBTAINED BY GINSBURG AND HELLER (22), SEE TEXT

mone (vasopressin) and the renal tubular reabsorption of water in continuously water-loaded young adult men. The vasopressin was infused at various constant rates, almost all of which were less than that required to produce an hypertonic urine. The difference between the clearance of free water during maximal water diuresis and the clearance of free water during an infusion of vasopressin (ΔC_{H_2O}) was considered to represent the rate of reabsorption of water resulting from the particular rate of infusion of vasopressin. The results indicate that there is a graded curvilinear relationship between the rate of infusion of vasopressin and the renal tubular reabsorption of water. The relationship can be expressed by at least three simple regression equations, the possible physiologic significance of which has been discussed. It is suggested that an equation of the form $1/\Delta C_{H_2O} = A + B$ (1/vasopressin rate) may be the most physiologically meaningful expression of the data.

ACKNOWLEDGMENTS

The authors wish to thank Mr. Edmund Gehan of the Department of Biostatistics, Dr. Harry Smith, formerly of the same department, and Dr. Robert Mann of the Department of Mathematics, all of the University of North Carolina and all of whom gave considerable assistance with statistical and mathematical considerations.

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APPENDIX A

Derivation of equation based on "pore" theory of Ussing (13). Let:

H = Available ADH molecules.
M_0 = Total number of "sites" potentially able to combine with ADH to form "pores."
M = Number of "sites" not combined with ADH.
HM = Number of "pores" (H and M combinations).
ΔW = Rate of reabsorption of water resulting from ADH.
a = Constant.

Assume:

1) That combination of H and M is reversible and obeys the law of mass action;
2) That ΔW is proportional to the number of "pores."

Then:

$$M_0 = M + HM \quad \text{(I)}$$

$$H + M \rightleftharpoons HM \quad \text{(II)}$$

$$\frac{H \times M}{HM} = k \quad \text{(III)}$$

$$\Delta W = a\,(HM) \quad \text{(IV)}$$

Substituting from (I) and (IV) in (III):

$$\frac{H\left(M_0 - \frac{\Delta W}{a}\right)}{\frac{\Delta W}{a}} = k \quad \text{(V)}$$

Rearranging (V):

$$\frac{1}{\Delta W} = \frac{1}{M_0 a} + \frac{k}{M_0 a}\left(\frac{1}{H}\right) \quad \text{(VI)}$$

APPENDIX B

Let:

H = Available ADH molecules.
ΔW = Rate of reabsorption of water resulting from ADH.
Δ osm = Increased osmolality of renal tubular cells resulting from ADH.
a = Effective osmolality of renal tubular cells in absence of ADH = constant.
b = Rate at which renal tubular urine is delivered to site where ADH is effective = constant.
k = Constant.

Assume:

1) That renal tubular urine will always become isosmotic with the renal tubular cells = a + Δ osm;
2) That the rate of reabsorption of solutes is constant;
3) That Δ osm is proportional to H.

Then:

$$\Delta W = b - \frac{ab}{a + \Delta\,\text{osm}} \quad \text{(I)}$$

Rearranging (I):

$$\frac{1}{\Delta W} = \frac{1}{b} + \frac{a}{b}\left(\frac{1}{\Delta\,\text{osm}}\right) \quad \text{(II)}$$

From assumption (3):

$$\frac{1}{\Delta W} = \frac{1}{b} + \frac{ak}{b}\left(\frac{1}{H}\right) \quad \text{(III)}$$

ACUTE RESPIRATORY ILLNESS IN VOLUNTEERS FOLLOWING INTRAMUSCULAR ADMINISTRATION OF LIVE ADENOVIRUS

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Early investigations of the pathogenicity of the adenoviruses (RI–APC–ARD agents) (1–5) for man revealed (3, 6, 7) that volunteers given tissue culture-grown virus by respiratory, intradermal, or intramuscular routes regularly developed neutralizing and complement-fixing antibodies against the agents without significant illness. This finding led us to attempt to prepare live virus vaccine for human immunization employing types 4 and 7 adenovirus grown in tissue cultures of monkey kidney. When it was found (8, 9), however, that monkey kidney cultures may contain "wild" viruses of unknown pathogenicity for man, the idea of using such untreated monkey kidney material in volunteers was abandoned and a formalin-killed vaccine was prepared instead. The high degree of effectiveness of the killed vaccine in preventing acute respiratory illnesses caused by adenovirus in newly inducted soldiers has been reported elsewhere (10–12).

Continued investigation of the live virus vaccine problem was made, employing types 3, 4, and 7 adenovirus produced in tissue cultures of human embryo intestine which had been propagated serially *in vitro*. Contrary to expectation, volunteers given the trivalent live virus preparation intramuscularly developed acute febrile respiratory illness which resembled closely the natural disease caused by these agents. This report describes the experimental illness induced in the volunteers and presents the antibody responses in these individuals.

MATERIALS AND METHODS

Preparation of virus for human injection

Tissue cultures of human intestinal cells. The virus injected into the volunteers was propagated in human embryonic intestinal cells of non-malignant origin. This cell line was initiated by Dr. Gertrude Henle and her associates (13) from a plasma clot explant culture of human embryonic small intestine grown in nutrient medium containing human serum. The cells originally propagated in tissue culture grew slowly and resembled fibroblasts and histiocytes. In the fourth passage, epithelial-like cells appeared which rapidly overgrew the culture and were predominant thereafter. The origin of the epithelial-like cells is unknown but these may have been present from the start, having been obscured by the more numerous fibroblasts, or they may have been derived by "transformation" from some previously existing cell type.

After 20 passages in Dr. Henle's laboratory, the culture was sent to Microbiological Associates,[2] Bethesda, Maryland, where it was passed in a medium consisting of Eagle's basal medium with 10 per cent horse serum. In each passage the culture fluid was removed, the cell layer digested with trypsin solution and the cells planted in fresh horse serum nutrient. Tissue cultures employed to grow the adenoviruses had been passed 16 to 23 times in the horse serum nutrient. For virus propagation, these cultures were washed five times with synthetic mixture 199 to remove the serum nutrient and were maintained in this solution with 100 units of penicillin and 100 μg. streptomycin per ml.

Virus. The adenovirus type 3 strain RI–55–33 and type 4 strain RI–55–32 were recovered in cultures of human intestine from the throat washings of military patients with undifferentiated acute respiratory disease (ARD) (14). Type 7 strain RI–4–202 was recovered in human amniotic epithelium culture from throat washings of a patient with ARD and, after one additional passage in such cells, was propagated in cultures of human intestine. These viruses grew abundantly in human intestine cultures causing total degeneration in 24 to 48 hours resembling that described (1) for the HeLa strain of human epidermoid carcinoma cells.

To prepare virus for human injection, 32-oz. prescription bottle cultures of human intestine, maintained with 25 ml. of mixture 199 containing antibiotics, were inoculated each with 1 ml. of undiluted seed virus. After 2 days' incubation at 36° C., the whole cultures were homogenized for 3 minutes in a Waring blendor and the supernatant fluid obtained following low speed centrifugation was further clarified by filtering through a sintered glass filter of medium porosity. A sample of each filtrate was saved for individual testing. A pool comprising equal volumes of the types 3, 4, and 7 viruses was prepared

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[2] All tissue cultures and tissue culture media employed in this investigation were obtained from Microbiological Associates, Bethesda, Maryland.

for human use and stored frozen at − 70° C. in sealed glass ampoules until injected into volunteers 2 months later.

Safety, identity, and sterility tests. Tests for safety and sterility of the trivalent virus pool were carried out in suckling and adult mice, guinea pigs, rabbits, monkeys, and a variety of artificial media as described previously (10, 11) for the formalin-killed bivalent adenovirus vaccine. Additionally, 2 serial passages of the virus in 6- and 10-day chicken embryos by the yolk sac and allantoic routes were carried out; the embryos remained normal and no hemagglutinins for human "O" or chicken red blood cells were present in the allantoic fluids. Neutralization tests using monotypic rabbit antisera were carried out in HeLa and monkey kidney cell tissue cultures as described earlier (1); these served to identify the virus in the monovalent filtrates and showed the absence of other detectable cytopathogenic agents.

Virus laboratory tests

Virus recovery attempts from patients' throat washings or eye swabs were carried out in duplicate in HeLa and human intestine tissue cultures by the same general methods described earlier (1, 10). Passage of the triturated whole cultures was made at 5- to 7-day intervals and from 3 to 7 such serial passages of each isolation attempt were made. Each eye specimen was tested one time in HeLa and human intestine cell cultures and each throat washing from 2 to 5 times in HeLa and 2 or 3 times in human intestine cells.

Serology. The methods for the complement-fixation (C.F.) and neutralization tests were described previously (1). Type 4 strain RI-67 virus grown in HeLa cell tissue culture was employed for C.F. antigen and types 3, 4, and 7 strains RI-3-110, RI-67, and RI-4-202 (2), respectively, propagated in HeLa cultures were used to measure neutralizing antibody. All serum titers are expressed as the greatest initial dilution of serum which caused complete or nearly complete fixation of complement or of suppression of virus growth (neutralization).

Human volunteers. The human volunteer study described here was intended primarily to be an additional test of safety to detect hepatitis virus which might have been carried—though only a remote possibility—in the human intestine cell line used to propagate the adenoviruses. Five of the six volunteers were adult male civilian employees of the Walter Reed Army Institute of Research and the other was a commissioned officer assigned to the Institute. All the volunteers were fully aware of the possible dangers involved. No epidemic of acute respiratory illness occurred in the personnel of Walter Reed Army Institute of Research either immediately preceding the time of the injection of virus or during the following 3-week period. Additionally, tests of the pre-inoculation and 2-week post-injection sera from each volunteer showed no diagnostic increase in hemagglutination-inhibiting antibody against type A prime (FLW-1-52) or type B (Lee and IB1) influenza viruses.

Prior to injection, each volunteer was given a chest X-ray and a detailed hospital admission type of physical examination with special attention to inspection of the eyes, ears, nose and throat. All volunteers were in good health at the time of inoculation. Daily clinical observations of the volunteers were made during the first 9 days and periodically thereafter for 6 to 8 months. Samples of blood, for serological testing, and throat washings and conjunctival swabs, for virus recovery attempts, were taken at the time periods indicated in the clinical charts. The specimens were stored frozen in sealed containers at − 20° C. or at − 70° C. until examined.

TABLE I

Composition of trivalent adenovirus preparation

Virus		Human tissue culture passage		Titers	
Type	Strain	Amnion	Intestine	Infectivity	C.F.
3	RI-55-33	0	9	10^{-3}	1:16
4	RI-55-32	0	8	10^{-3}	1:4
7	RI-4-202	2	7	10^{-3}	1:8

RESULTS

Trivalent live virus pool

The composition of the types 3, 4, and 7 adenovirus pool injected into the volunteers is shown in Table I. The infectivity titer of each strain was 10^{-3} and the C.F. titers ranged from 1:4 to 1:16. The total nitrogen value for the pool was 0.213 mg. per ml. and the protein nitrogen content was 0.024 mg. per ml.

The six volunteers in the study were each given 1 ml. of the trivalent live virus preparation at approximately 4:00 P.M. on day 0. Unexpectedly, in view of previous experience (3, 6, 7), five of the six volunteers developed an acute upper respiratory illness which resembled that described for ARD (14, 15) with conjunctivitis (16) and for pharyngoconjunctival fever (17). Basically the same illness occurred in each case but the degree of severity differed markedly. Three patients (cases 1, 2, and 3) had a moderately severe illness while two (cases 4 and 5) developed a mild form of disease. Case 6 also developed respiratory symptoms but these were so mild that they might not have been recognized had they not been searched for. Three of the patients, two with severe and one with mild illness, experienced a moderately severe primary toxic reaction shortly following injection of virus. The clinical picture and the laboratory findings in each of the patients are described in the summaries which follow.

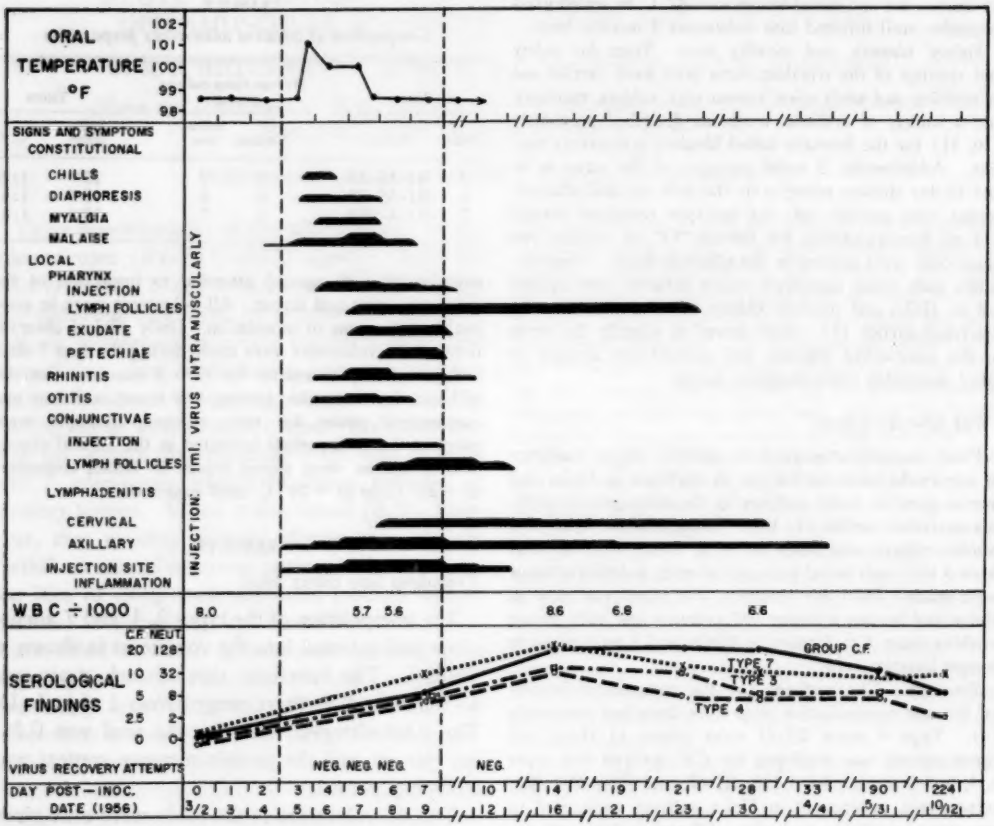

FIG. 1. CASE 1, CLINICAL AND LABORATORY FINDINGS

Case summaries

Patient 1, Figure 1. On the third day after inoculation, this 39-year-old man, who was previously well, complained of feeling listless and of soreness of the injection site. Late the same evening there was an abrupt onset of constitutional symptoms which consisted of generalized aching of the back, arms, and legs followed shortly by chills, sweating, and a temperature rise to 101° F.

The patient continued to feel ill on the following day at which time the localized signs and symptoms began. The patient complained of generalized aching, pain in the left ear, coryza and a sore throat. On physical examination, the conjunctivae were found to be injected and the left tympanum was erythematous and retracted. The nasal mucosa was inflamed and edematous and there was a watery discharge. The pharynx was also edematous and acutely inflamed. The site of injection was swollen, erythematous, indurated, and painful and the corresponding axillary nodes were markedly enlarged and very tender. The acute illness continued through the fifth day when the patient complained of generalized aching, fatigue, and excessive sweating. The inflammation of the conjunctivae, ears, and nasal mucosa had increased. Hyperplastic lymph follicles appeared on the posterior wall of the intensely inflamed pharynx and an easily removable whitish exudate of "pin-head" size surmounted the surface of many of these follicles. A few enlarged sublingual nodes were also present. The site of injection was elevated, intensely inflamed and swollen, involving an area of erythema of 8 cm. diameter and the corresponding axillary nodes were now 2 to 3 cm. in size.

Recovery from the acute illness began on day 6 when the temperature returned to normal. The conjunctivitis, however, had not diminished and the right eye at this time revealed intense injection and suffusion of the bulbar and palpebral conjunctivae. Lymphatic hyperplasia produced elevations of the mucosal surface. Inflammation of the ear, nose, and throat had subsided but petechiae were noted about the base of the uvula. The pharyngeal follicles were increased in size and exudate was present on most of these elevations. Sublingual nodes were prominent. The injection site revealed increased

inflammation, induration, and tenderness but was not fluctuant. The axillary nodes were enlarged bilaterally but those corresponding to the injection site were larger and more tender. Recovery was uneventful and was characterized by rapid diminution of the inflammation with a more gradual reduction of the lymphatic hypertrophy. No further illness or significant physical changes occurred in the patient during the remaining 7 months of observation.

Chest X-rays made prior to and 7 days post-inoculation were negative. The total and differential white blood cell counts remained within normal limits throughout the observation period. Serum bilirubin levels, performed to detect hepatic dysfunction, were within normal limits throughout the study period. Cultures of the throat made on the fourth and sixth days during the acute illness revealed a normal bacterial flora. Adenovirus could not be recovered from the eye on day 6 or from the throat on days 4, 5, 6, or 10. There was no detectable neutralizing antibody against types 3, 4, or 7 virus in the pre-inoculation serum specimen but antibody to all 3 types was already present on day 7 when the illness was in the acute inflammatory stage. Maximal titer levels were reached by the fourteenth day.

Patient 2, Figure 2. Seven hours following injection of virus, this 39-year-old man who was previously well experienced an abrupt onset of constitutional symptoms characterized by shaking chills with fever up to 103° F., sweating, and generalized aching of the back and extremities. This was accompanied by nausea and one episode of vomiting. There were no localizing signs and the intermittent fever and constitutional symptoms subsided by the third day. On day 4 the temperature rose again to 101.2° F. and ushered in the acute respiratory illness. The patient complained of a sore throat, nasal discharge, and mild anorexia. The conjunctivae of both

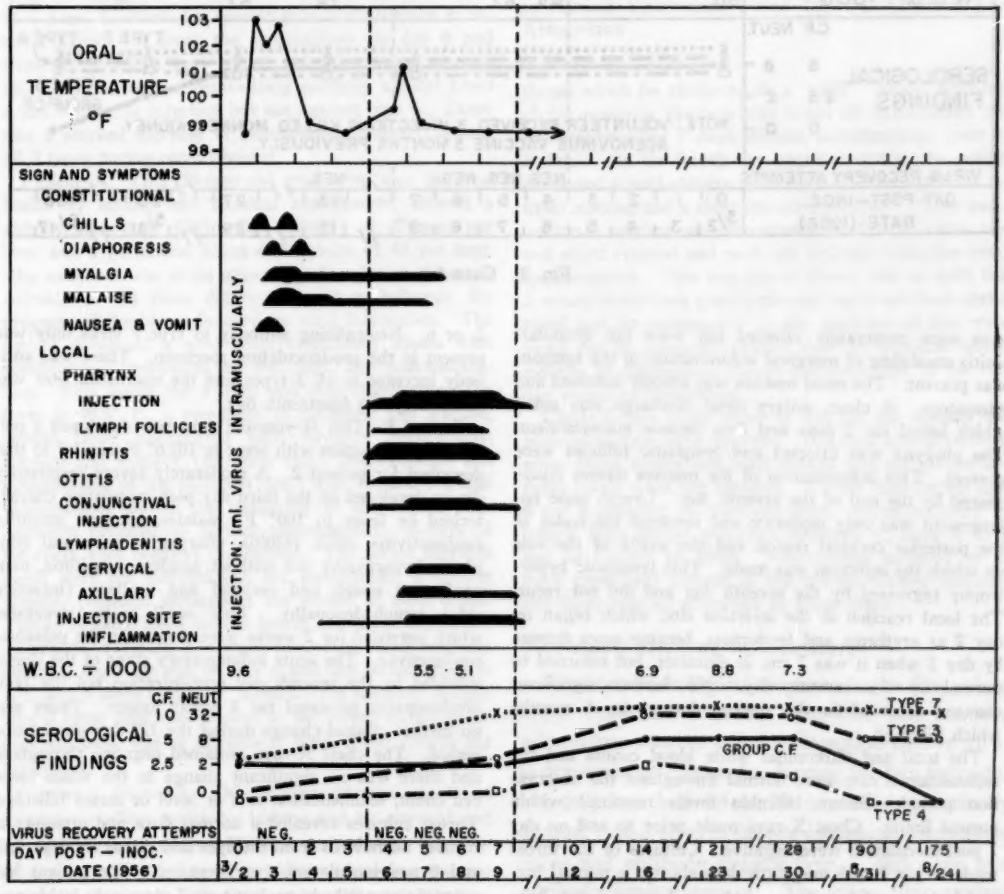

FIG. 2. CASE 2

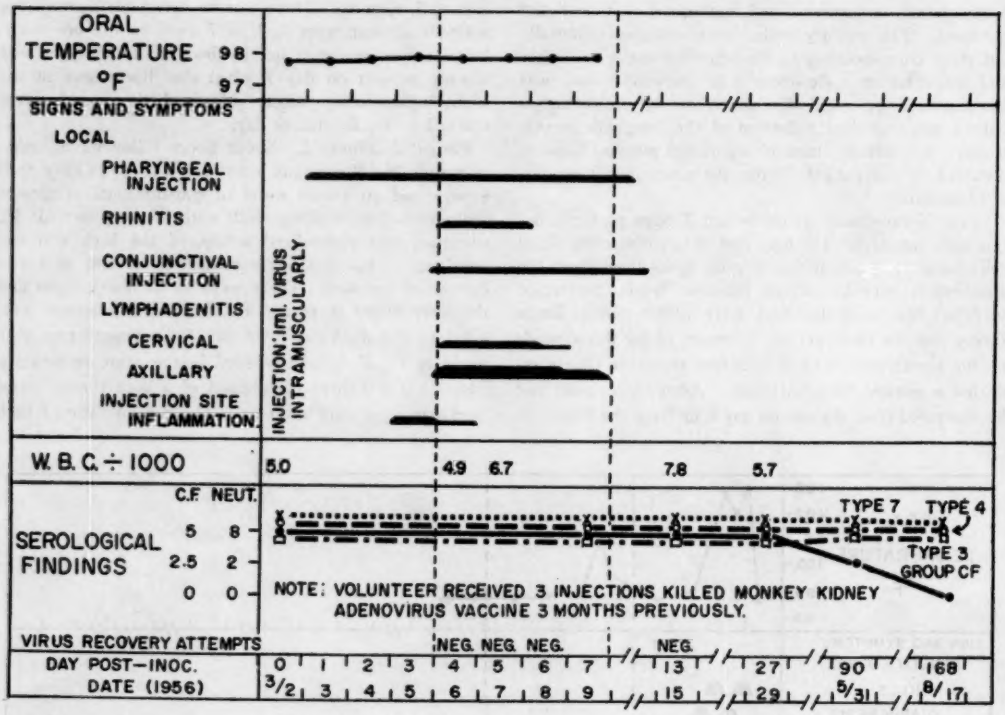

FIG. 3. CASE 6

eyes were moderately injected but were not granular. Otitis consisting of marginal inflammation of the tympani was present. The nasal mucosa was acutely inflamed and edematous. A clear, watery nasal discharge was noted which lasted for 2 days and then became mucopurulent. The pharynx was injected and lymphatic follicles were present. This inflammation of the mucous tissues disappeared by the end of the seventh day. Lymph node enlargement was only moderate and involved the nodes of the posterior cervical region and the axilla of the side on which the injection was made. This lymphatic hypertrophy regressed by the seventh day and did not recur. The local reaction at the injection site, which began on day 2 as erythema and tenderness, became more intense by day 5 when it was 7 cm. in diameter, but returned to normal by the seventh day. No further significant changes occurred in the patient during the 6 months which followed.

The total and differential white blood counts and the sedimentation rate were normal throughout the observation period. Serum bilirubin levels remained within normal limits. Chest X-rays made prior to and on day 7 post-inoculation were negative. Cultures of the throat made on the fourth and sixth days showed a normal bacterial flora. Adenovirus could not be recovered from the throat washings of the patient collected on days 1, 4, 5, or 6. Neutralizing antibody to type 7 virus only was present in the pre-inoculation specimen. There was antibody increase to all 3 types and the maximum titer was reached by the fourteenth day.

Patient 3. This 31-year-old volunteer developed a primary toxic reaction with fever to 102.6° F. similar to that described for patient 2. A moderately severe respiratory illness developed on the third day post-inoculation characterized by fever to 100° F., malaise, bilateral granular conjunctivitis, otitis, rhinitis, pharyngitis with oral lymphatic hypertrophy but without exudate, tonsillitis, nonproductive cough and cervical and axillary (injection side) lymphadenopathy. Two small cystic structures which persisted for 2 weeks were present in the palpebral conjunctivae. The acute inflammatory stage of the illness subsided by the seventh day post-injection but the lymphadenopathy persisted for 2 weeks longer. There was no further clinical change during the 172-day observation period. The chest X-rays remained negative throughout and there was no significant change in the white blood cell count, sedimentation rate or level of serum bilirubin. Throat cultures revealed a normal flora and attempts to recover adenovirus from the eyes and throat on days 4, 5, and 6 post-inoculation were negative. The patient had neutralizing antibody against type 7 virus only in his pre-inoculation specimen. Marked antibody increase against

all 3 types occurred and this reached the maximum level by the time the acute inflammatory stage of the illness had subsided.

Patient 4. This 28-year-old man developed a severe primary toxic reaction to the virus inoculum which began 5 hours post-injection and resembled that described for patient 2. An episode of mild acute respiratory illness which followed on day 4 was characterized by a granular conjunctivitis, rhinitis, and pharyngitis with lymphatic hyperplasia in the pharynx. Constitutional symptoms were absent. The patient did not feel feverish and his temperature was not taken. The injection site was moderately inflamed. Mild cervical and axillary (injection side) lymphadenopathy were present on day 5 and lasted about 3 weeks. There was no further significant change during the remainder of the 182-day observation period. The chest X-rays were negative throughout and there was no significant alteration in either the total and differential white blood cell counts or in the serum bilirubin level. Throat cultures taken on days 4 and 6 revealed a normal flora and attempts to recover adenovirus from the conjunctivae on day 6 and from the throat on days 4, 5, 6, and 10 were not successful. The patient had neutralizing antibody against types 3 and 7 prior to injection but not against type 4. There was a marked increase in neutralizing antibody against all 3 types during convalescence.

Patient 5. This 36-year-old man who was in good health by history and physical examination, showed a sedimentation rate (Wintrobe method) of 27 mm. per hour and a peripheral blood eosinophilia of 40 per cent. The volunteer was given adenovirus in spite of these abnormalities and these did not appear to influence the progress of the illness induced by viral inoculation. The patient developed a mild acute respiratory illness with constitutional symptoms on the third day post-inoculation. This lasted for 5 days and was characterized by fever to 99.8° F., a complaint of chilliness and malaise, rhinitis, mild pharyngitis with tonsillar hypertrophy, cervical and axillary (injection side) lymphadenitis and inflammation of the injection site. The routine physical examinations during the remaining of the 171-day observation revealed no further abnormalities. X-rays of the chest were negative throughout. The serum bilirubin levels were not elevated and the eosinophilia, originally present, persisted throughout the period of observation. Throat cultures made on the fourth and sixth days post-inoculation revealed a normal flora and virus recovery attempts from the eye on day 6 and the throat on days 4, 5, and 6 were negative. Neutralizing antibody against type 3 virus only was present in the initial serum specimen and there was an increase to types 3 and 7 virus but not type 4.

Patient 6, Figure 3. This 36-year-old man was found to be in excellent health by history and by physical and laboratory examinations. He had received three 1-ml. injections of the killed monkey kidney adenovirus vaccine 3 months previously. On the first day following administration of the live adenovirus he developed a mild sore throat which he attributed to a "cold." Slight erythema of the posterior pharynx was noted on examination and this persisted for 7 days without accompanying fever or malaise. On the fourth day post-inoculation, the patient developed a mild rhinitis with erythema, congestion of the nasal mucosa, and a clear mucoid discharge. There was a moderate amount of inflammation at the injection site, and slight cervical and moderate axillary (injection side) lymphadenitis. This non-febrile illness was so mild that it would likely have gone unnoticed had it not been anticipated and its relationship to the injection of live virus could not be assessed. There were no physical abnormalities of significance during the remainder of the 166-day period of observation.

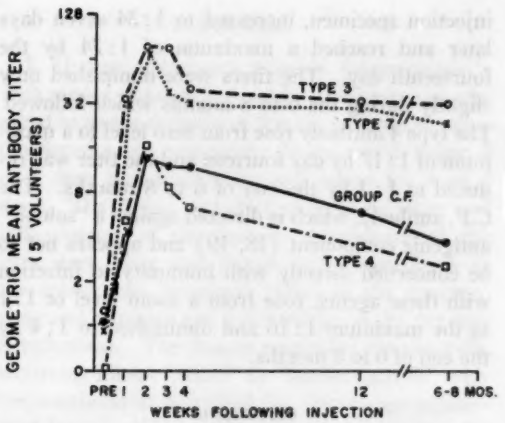

FIG. 4. NEUTRALIZING AND C.F. ANTIBODY PATTERNS IN HUMAN VOLUNTEERS GIVEN TRIVALENT POOL OF LIVE ADENOVIRUS

Chest X-rays made prior to and on the seventh day post-inoculation were negative. The total and differential white blood counts remained normal and the serum bilirubin levels were not elevated. Throat cultures on days 4 and 6 revealed no abnormal flora. Virus recovery attempts from throat washings collected on days 4, 5, 6, and 13 yielded no virus. Neutralizing antibody to all three types was present in the pre-inoculation specimen and there was no alteration in these titers.

Serological response in volunteers

The mean serological response in the five volunteers (patients 1 to 5) who had not received adenovirus vaccine prior to injection of live virus is presented in Figure 4. Among the five individuals, only two had detectable neutralizing antibody against type 3 virus in the pre-inoculation specimen, three had antibody against type 7, and none had antibody against type 4. The mean titer against types 3 and 7, which was about 1:1 in the pre-

injection specimen, increased to 1:34 seven days later and reached a maximum of 1:74 by the fourteenth day. The titers were diminished only slightly during the 6 to 8 months which followed. The type 4 antibody rose from zero level to a maximum of 1:17 by day fourteen and the titer was reduced to 1:3 by the end of 6 to 8 months. The C.F. antibody, which is directed against a "soluble" antigenic component (18, 19) and appears not to be concerned directly with immunity to infection with these agents, rose from a mean level of 1:1 to the maximum 1:16 and diminished to 1:4 by the end of 6 to 8 months.

DISCUSSION

Previous workers have reported the development of acute respiratory illness in human volunteers inoculated nasally with the filtered throat washings from a patient with ARD (4, 20, 21) and in human beings inoculated by the conjunctival or nasal route with adenoviruses propagated in tissue culture (22, 23). The present experiments represent the first successful reproduction of acute respiratory disease in persons given adenovirus by a parenteral route and strongly support the etiological significance of these agents in respiratory illnesses of man. Earlier experiments (3, 6, 7), in which adenoviruses were given parenterally by the intramuscular, intracutaneous, and intratumor routes, and more recently (24), intravenously, presented evidence of viral infection but without significant illness. The reason for the present experience, in contrast to the earlier, has not been determined but differences in the viral inocula employed may be of significance. The adenoviruses employed heretofore were grown in tissue cultures of human epidermoid carcinoma or monkey kidney, while the present virus was propagated in human intestine cell cultures. Further, the infectivity titer of the present inoculum was 10 times as great as that employed before (7) and three types of virus were injected simultaneously instead of a single type.

The clinical course which followed injection of the trivalent virus pool was in three phases. The initial phase, which lasted 2 or 3 days, was the incubation period. During this period, three of the volunteers experienced a febrile toxic reaction with constitutional symptoms. This is believed to have been due to toxicity of the viral inoculum and to be unassociated with infection. The second phase, which began 3 or 4 days post-inoculation and lasted for 4 or 5 days, represented the stage of acute respiratory illness. The third phase was the period of convalescence. The acute respiratory illness induced by adenovirus resembled closely that described by Dascomb and Hilleman (16) for laboratory-proved cases of the natural disease, and also the previous clinical descriptions of febrile catarrh (25) and of ARD (14, 15) with conjunctival inflammation such as occurs in pharyngo-conjunctival fever (7). Thus, there were fever, constitutional symptoms, and inflammation of the mucosae of the eyes, ears, nose and throat accompanied by characteristic lymphoid hyperplasia in the pharynx and conjunctivae. Exudate, such as commonly occurs in natural adenovirus disease (16), was also present on the hyperplastic lymphatic tissue in the throat of case 1. Cervical lymphadenopathy was a common feature of both the natural and experimental disease. None of the volunteers, however, presented signs or symptoms indicative of spread of the inflammatory process to the larynx, trachea or the lower respiratory tract such as occurs in naturally-acquired adenovirus infections. The 2 or 3-day incubation period of the disease was consistent with the 2 to 7-day period reported by other workers who employed different kinds of adenovirus preparations given by different routes (20–23).

The failure to recover adenovirus from the eyes and throats of the volunteers was not referable to the isolation techniques employed since adenoviruses were concurrently recovered, in the same laboratory, from 44 of 55 serologically proved cases of naturally occurring adenovirus disease (12). Instead, the negative recovery attempts clearly excluded the presence of detectable virus in the conjunctival and nasopharyngeal secretions of the volunteers during their illness. This absence of virus from the secretions might be related to the intramuscular route of infection in contrast to the natural route which is presumed to be via the respiratory tract and possibly the conjunctivae. Of greater probable significance was the rapid increase in amount of neutralizing antibody in the volunteers against all three virus types which had achieved the maximal or near maximal level by the fourth or fifth day after onset of illness.

Such a circumstance seems incompatible with the excretion of significant amounts of viable virus and might account also for the relatively mild form of the illness in the volunteers. This rapid antibody increase is in striking contrast to the natural infection in which the homologous neutralizing antibody is absent or of very low level during the acute illness and does not usually attain the maximum titer until 3 weeks after onset of the disease. Indeed, this pattern resembles the rapid antibody response which follows use of killed monkey kidney adenovirus vaccine (10, 11), in which there is marked increase after 1 week reaching maximum level by 2 weeks post-inoculation, and strongly suggests that the trivalent material given the volunteers acted as a live virus challenge dose combined with a sufficient amount of viable or non-viable virus to permit immediate stimulation of the antibody mechanism.

Live adenovirus vaccine presents the same potential and theoretical advantages as may be expected for living vaccines against other diseases such as poliomyelitis, influenza, rabies, yellow fever, typhus and others. Thus, the immunity induced by live virus might be broader immunologically and less type or strain specific, may be longer lasting, and a much smaller quantity of virus may be needed to achieve the desired effect. The last mentioned factor may be of great importance for the adenovirus vaccine since, according to present estimates, the killed monkey-kidney vaccine may prove expensive to produce and assay. Likewise, employment of living vaccine may be of advantage in young children in whom prior experience with these agents may be lacking and the recall phenomenon consequently non-operative.

The 1 ml. of undiluted viral inoculum given the volunteers, which represents a large dose of adenovirus, was employed for the purpose of providing an adequate test dose for detection of possible infectious hepatitis A or B virus. It is believed that appropriate reduction in amount of virus given might, as found in previous experiments (3, 6, 7), have stimulated antibody formation without causing significant clinical illness. The high degree of effectiveness of the killed monkey kidney vaccine (10–12) and the theoretical objections to the use in man of virus grown in serially propagated cells of human origin have led us to delay further tests of the human intestine preparation in man.

SUMMARY

1. Human volunteers injected intramuscularly with a pool of types 3, 4, and 7 adenovirus propagated in tissue cultures of human embryo intestine developed acute respiratory illness which began 2 or 3 days post-inoculation and lasted for 4 or 5 days. The illness was characterized by fever and inflammation of the mucous membranes of the eyes, ears, nose and throat with hyperplasia of the submucosal lymphatic tissue of the conjunctivae and pharynx and hypertrophy of the cervical lymph nodes. The disease resembled the naturally occurring illness "ARD" or "febrile catarrh" with conjunctivitis as described for "pharyngoconjunctival fever." Three volunteers experienced a febrile toxic reaction which followed several hours after inoculation of virus.

2. All volunteers, except one who had previously received killed virus vaccine, showed a marked increase in neutralizing antibody against all three types of virus and in complement fixing antibody. The antibody titers were diminished, on the average, 3 to 6-fold by the end of the 6 to 8-month observation period.

3. The significance of the findings in relation to the reproduction of the disease following parenteral inoculation of virus and the use of live virus vaccine against adenovirus infections are discussed.

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PULMONARY VENTILATION AND DIFFUSION IN THE HUMAN NEWBORN INFANT[1]

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Respiratory failure is the most common cause of morbidity and mortality in the first few days of life, particularly in the premature infant. However, the pathologic physiology of both internal and external respiration in this age group is poorly understood.

The primary obstacle to the definition of normal and altered respiratory physiology in the newborn has been the lack of practical methods for studying ventilation, diffusion and perfusion of the lungs in the very young infant. Techniques for such studies in coöperative adults are, with certain accepted limitations, well established as adequate means of quantitative measurement. Because of the marked differences in the size of the subjects, the volumes of gases to be measured, and the problems of resistance to expiration and dead space which arise when such equipment is used in small infants, these techniques have not previously been applied to the study of infants. Indirect methods, using a body plethysmograph (1–9), have allowed the estimation of some of the elements of ventilation, but cannot be applied to measurements of diffusion or distribution of inspired air.

The purpose of this paper is to describe equipment and techniques designed to allow direct quantitative measurement of ventilation and diffusion in newborn infants. Results of studies obtained by these techniques in a series of normal newborn infants are compared with similar measurements made by accepted techniques in older children and adults.

METHODS

The problems of measuring resting pulmonary ventilation in any age group depend upon the ability to measure the amount of gas respired in a one-way system with a simultaneous recording of respiratory frequency. The estimation of the diffusing properties of the pulmonary alveolar-capillary membrane has been approached by attempting to measure the diffusion capacity for a given gas. The diffusion capacity for a gas is defined as that quantity of a gas transferred each minute for each millimeter of mercury difference in partial pressure of that gas across the alveolar-capillary membrane (10). Because of the technical difficulties of applying to infants methods in current use for measuring the diffusion capacity of oxygen, and the many assumptions which have to be made before it can be considered a valid measurement (11, 12), the steady-state carbon monoxide method of Filley was chosen as a measure of the diffusion properties of the lung (13). Since carbon monoxide, when breathed in low concentrations (0.1 to 0.2 per cent), combines so readily with hemoglobin, the mean effective pulmonary capillary partial pressure of carbon monoxide can be kept essentially zero and may be ignored as a measurement in this method (14, 15). The method requires, therefore, only a knowledge of carbon monoxide uptake and alveolar partial pressure of carbon monoxide.

Because of the necessity of keeping the apparatus dead space and resistance to expiration at a minimum in the measurement of carbon monoxide diffusion capacity and pulmonary ventilation in newborns, a double Donald-Christie box arrangement was chosen with a 500-ml. counter-balanced spirometer interposed between the boxes (16, 17). A small tightly-fitting contour mask of lucite, molded to fit the death mask of a premature baby, with

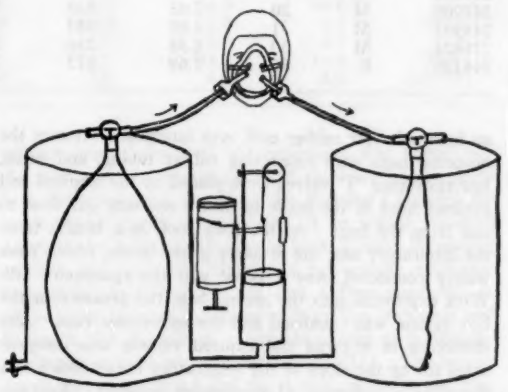

FIG. 1. DOUBLE DONALD-CHRISTIE BOXES WITH A SMALL COUNTER-BALANCED SPIROMETER INTERPOSED BETWEEN THE BOXES

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TABLE I

Data used in calculating the diffusion capacity in infants

Number	Sex	Age (*hr.*)	Wt. (*lb.*)	CO uptake (*ml./min.*)	pH (art.)	CO_2 content (*mM/L. plasma*)	P_ACO_2 (*mm. Hg*)	P_ACO (*mm. Hg*)	D_{CO} (*ml./min./mm. Hg*)
240177	F	81	8.31	.334	7.43	23.18	33.0		
240316	M	29	6.50	.270	7.42	16.32	23.8		
240316	M	72	6.19	.230	7.47	16.03	20.9		
240408	M	24	6.75	.207	7.39				
230465	M	50	6.75	.347	7.46	15.55	20.7		
240696	M	12	6.94	.276	7.50	22.26	27.1	.146	1.89
240960	F	48	7.03	.234	7.44	20.60	28.7	.086	3.10
241058	F	54	7.50	.240	7.41	17.48	26.0	.234	1.03
241221	F	75	5.82	.143	7.48	23.10	29.5		
241259	M	30	9.05	.196	7.42	22.48	32.7	.159	1.24
241356	M	30	5.69	.166	7.43	23.30	33.2	.138	1.20
241362	F	96	6.82	.261	7.46	19.84	26.6		
241469	F	52	7.69	.277	7.39	20.91	33.2	.231	1.14
241614	F	30	6.10	.189	7.43	19.26	27.4	.218	0.87
241681	M	29	7.75	.218	7.41	21.91	32.6	.222	0.98
241758	M	81	7.69	.260	7.47	24.10	31.8		
241877	M	80	7.31	.244	7.46	19.94	26.6	.223	1.09
242026	F	34	8.57	.227	7.45	23.20	31.6	.129	1.76
242068	F	40	5.75	.330	7.45	23.04	31.4	.056	
242510	F	58	8.10	.322	7.43	27.98	39.8	.047	
242570	M	40	6.81	.253	7.40	23.32	35.4	.234	1.08
242574	M	53	6.78	.274	7.43	23.03	32.8	.130	2.11
242676	F	64	7.38	.316	7.38	32.48	29.7	.246	1.28
242811	F	22	8.25	.190	7.45	23.08	31.4	.201	0.94
242861	M	30	8.19	.282	7.42	22.39	32.6	.168	1.68
242971	M	48	7.31	.237	7.42	23.61	34.4	.088	2.69
243519	F	63	7.81	.172	7.44	26.42	36.8		
243651	M	9	7.22	.211	7.39	21.18	32.9	.130	1.62
243771	F	53	6.50	.252	7.44	20.70	28.8	.184	1.37
243933	F	64	5.56	.188	7.41	21.64	32.2	.235	0.80
244135	M	20	7.88	.266	7.44	21.35	29.7	.214	1.24
244194	M	32	7.63	.250	7.43	23.10	32.8	.097	2.58
244570	F	13	6.13	.194	7.39	22.87	35.5	.180	1.08
244657	M	23	7.75	.250	7.44	20.87	29.0	.141	1.77
244779	F	36	7.38	.215	7.41	21.78	32.4	.218	0.99
244923	M	56	6.81	.176	7.46	21.60	28.8		
245086	F	13	8.25	.155	7.41	21.52	32.0	.101	1.41
247612	F	30	6.94	.156	7.42	21.53	31.3		
247533	M	78	6.08	.184	7.42	25.13	36.6	.110	1.68
247696	M	37	8.22	.310	7.44	20.16	28.1	.138	2.25
247909	M	20	7.05	.139	7.45	20.40	29.1	.065	2.14
248502	M	31	4.00	.083				.072	1.15
221824	M	30	8.38	.286	7.43	20.80	29.6	.105	2.72
248729	F	80	7.69	.172	7.50	19.95	24.3	.111	1.55

an inflatable soft rubber cuff, was interposed between the neoprene bags with connecting rubber tubing, and small, low resistance "J" valves were placed on the inspired and expired sides of the mask to insure one-way gas flow to and from the bags. As the baby took in a breath from the inspiratory bag, the pressure in the boxes, which were widely connected, was lowered and the spirometer fell. With expiration into the second bag, the pressure in the box system was equalized and the spirometer rose. Any difference in inspired and expired volume was compensated for by the slope of the respiratory curve which was therefore a reflection of respiratory quotient. Respiratory frequency could be read from the curve, inspired and expired gas concentrations analyzed separately without dilution, and the expired volume measured by emptying the expired bag into a nine-liter calibrated Collins spirometer. Figure 1 shows a diagram of the system. The inspired and expired carbon monoxide concentration were analyzed with a Liston-Becker Infra-Red Carbon Monoxide Meter set at full scale deflection with 0.1 per cent carbon monoxide, with an accuracy of ± 2 per cent of full scale. The expired and inspired oxygen, carbon dioxide, and nitrogen concentrations were measured with the Scholander Gas Analyzer, double determinations being required to check within 0.04 per cent. With this system, carbon monoxide uptake, minute ventilation, alveolar ventilation, oxygen uptake, and carbon dioxide output, frequency of respiration and tidal volume were obtained.

Arterial blood was collected anerobically in greased sterile 2-ml. syringes, the dead space of which had been filled with sterile heparin solution. The syringes were capped with mercury-seal caps and iced immediately. Arterial blood pH was done in duplicate on the Cambridge pH Meter at room temperature and corrected to

TABLE II

Data used in ventilatory calculations in infants

Number	M_V (*L./min.*) (BTPS)	f/min.	V_T (*ml.*)	V_D/V_T	V_A (*L./min.*) (BTPS)	O_2 uptake (*ml./min. STPD*)	CO_2 output (*ml./min. STPD*)	O_2 sat. (%)
240177	.63	55	11.6		.442	22.0	17.0	
240316	.50	42	11.9			18.0	13.2	98.3
240316	.44	45	9.9			17.0	12.9	85.4
240408	.39	46	8.4			17.0	11.9	
230465	.69	53	13.0			27.0	20.5	99.4
240696	.53	48	11.1	.143	.457	14.0	14.6	93.5
240960	.48	74	6.5	.265	.352	15.0	11.8	95.3
241058	.53	80	6.6	.142	.455	19.0	13.9	89.7
241221	.28	32	8.8			14.0	9.3	96.7
241259	.36	42	8.6	.113	.320	21.0	11.8	96.0
241356	.28	45	6.3	.034	.260	16.0	9.9	95.2
241362	.63	47	13.5			23.0	20.1	92.9
241469	.64	70	9.1	.178	.525	20.0	20.3	91.2
241614	.38	49	7.7	.069	.353	18.0	11.1	94.4
241681	.45	41	10.9	.107	.400	20.0	14.9	95.8
241758	.55	44	12.4			24.0	20.1	92.0
241877	.52	46	11.2	.119	.455	17.0	14.0	94.7
242026	.49	71	6.9	.294	.347	20.0	12.7	99.0
242068	.58	44		.232	.444	26.0	15.7	93.2
242510	.72	60	12.0		.434	24.0	20.0	96.3
242570	.57	57	10.0	.155	.483	23.0	19.8	90.8
242574	.58	73	8.0	.286	.417	24.0	15.7	94.1
242676	.76	62	12.2	.172	.627	27.0	21.6	94.2
242811	.47	44	10.6	.296	.330	17.0	11.9	94.9
242861	.64	74	8.6	.250	.478	21.0	18.3	96.9
242971	.44	46	9.5	.208	.347	18.0	13.9	89.6
243519	.28	38	7.5	.282	.201	13.0	8.6	85.4
243651	.42	47	8.9	.200	.336	15.0	12.9	82.2
243771	.51	56	9.1	.122	.448	18.0	14.9	97.8
243933	.44	44	10.0	.158	.371	14.0	13.8	90.8
244135	.65	49	13.3	.247	.489	20.0	17.1	91.1
244194	.51	59	8.6	.271	.372	17.0	14.0	97.3
244570	.42	62	6.8	.186	.342	17.0	14.1	97.6
244657	.54	59	9.1	.259	.400	19.0	13.5	98.4
244779	.54	64	8.4	.256	.402	20.0	15.0	96.7
244923	.32	41	7.8			12.0	11.0	94.0
245086	.28	37	7.6	.169	.233	14.0	8.5	88.7
247612	.27	74	3.6		.151	5.0	5.5	88.0
247533	.36	49	7.4	.239	.274	13.0	11.9	91.1
247696	.70	46	15.2	.287	.499	16.0	16.4	
247909	.22	48	4.6	.136	.190	7.0	6.6	97.7
248502	.15	35	4.3	.222	.117	4.0	4.7	
221824	.71	53	13.4	.401	.425	13.0	14.8	96.9
248729	.29	46	6.3	.114	.257	8.0	7.3	90.0

37° C. by subtracting 0.01 pH units for each degree below 37° C. Oxygen capacity, oxygen and carbon dioxide contents were run in duplicate on 0.2-ml. samples of whole blood on the Van Slyke Manometric Gas Apparatus. The Van Slyke and Sendroy line chart was used to calculate the plasma carbon dioxide content from that of whole blood (18).

All babies were from fifteen to forty-five minutes postprandial and most were sleepy or frankly asleep. A generous injection of Novocain® was administered into the right femoral triangle and this usually elicited one or two loud cries which rapidly subsided if the baby was soothed. A 20-minute wait followed during which time the baby was held on the operator's lap and rocked; then a femoral arterial puncture was attempted and, whenever possible, three 2-ml. syringes of blood obtained. If significant crying occurred the procedure was abandoned. As soon as the blood was obtained, the baby was wrapped in a sheet restraint, placed on the operator's lap and the mask placed gently on the face. If no crying occurred (usually the baby went promptly back to sleep if handled gently), the baby was allowed a two-minute equilibration period breathing 0.1 per cent carbon monoxide in air in order to wash the lungs with carbon monoxide and to insure that the carbon monoxide uptake to carbon dioxide output ratio was a constant one (13). The kymograph was then started and the 3-way valves on the two bags turned so as to allow the baby to breathe out of the first bag and into the second one. The first bag had been rinsed and filled with 0.1 per cent carbon monoxide in air, and the second one rinsed with room air and emptied to its dead space which had been previously meas-

TABLE III

The relationship of ventilatory and diffusion data to body weight in infants

		Body weight (*pounds*)				Correlation Coefficient	P value
		4.0 to 6.5	6.5 to 8.0	8.0 to 8.1	Total		
M_V, *L./min.*	N	8	26	9	43		
	Mean ± S.E.	0.34 ± 0.04	0.49 ± 0.03	0.58 ± 0.06	0.49 ± 0.02	+0.46	<0.01
V_A, *L./min.*	N	6	18	7	31		
	Mean ± S.E.	0.29 ± 0.04	0.41 ± 0.02	0.43 ± 0.05	0.39 ± 0.02	+0.41	<0.05
O_2 uptake, *ml./min.*	N	9	26	9	44		
	Mean ± S.E.	15.4 ± 1.9	17.7 ± 1.1	20.0 ± 1.7	17.8 ± 0.8	+0.31	<0.05
CO uptake, *ml./min.*	N	9	26	9	44		
	Mean ± S.E.	0.19 ± 0.02	0.24 ± 0.01	0.27 ± 0.02	0.23 ± 0.01	+0.39	<0.01
D_{CO}, *ml./min./mm. Hg*	N	6	18	7	31		
	Mean ± S.E.	1.13 ± 0.13	1.65 ± 0.15	1.70 ± 0.20	1.56 ± 0.11	+0.24	<0.25

ured as 20 ml. The baby was then allowed to breathe in the system for four minutes. If crying occurred the collection was discontinued.

The diffusion and ventilation measurements on the older children and adults were made by the method of Filley, MacIntosh, and Wright, using a one-way system of gas collection, and the same inspired mixture of carbon monoxide as that used for the babies (13). An indwelling arterial needle had been placed previously in a brachial or femoral artery, and the collection of blood was made simultaneously with the collection of expired gas.

Posterior-anterior and lateral X-ray films of the chest were also made on most of the subjects, both the

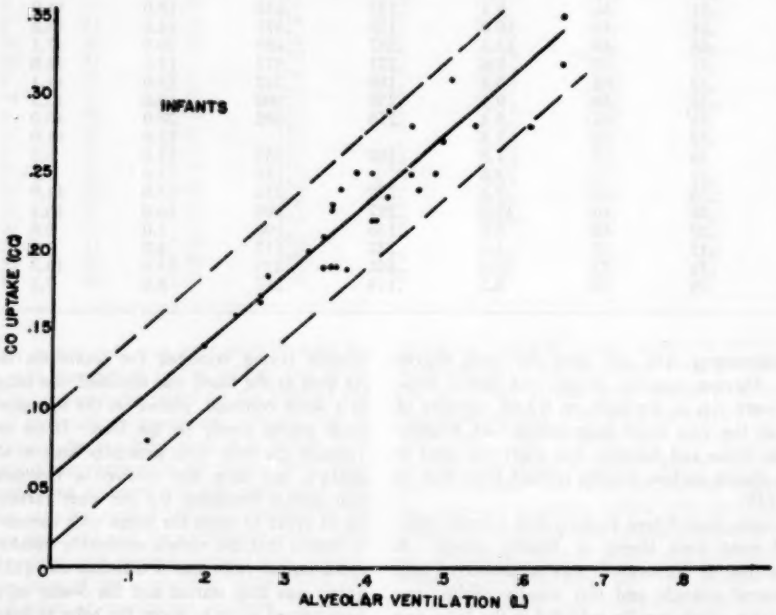

FIG. 2. THE CORRELATION OF CO UPTAKE IN ML. PER MINUTE WITH ALVEOLAR VENTILATION IN LITERS PER MINUTE IN 31 NORMAL NEWBORN INFANTS

The solid line represents the regression line for the data and the broken lines represent two standard deviations around the regression line.

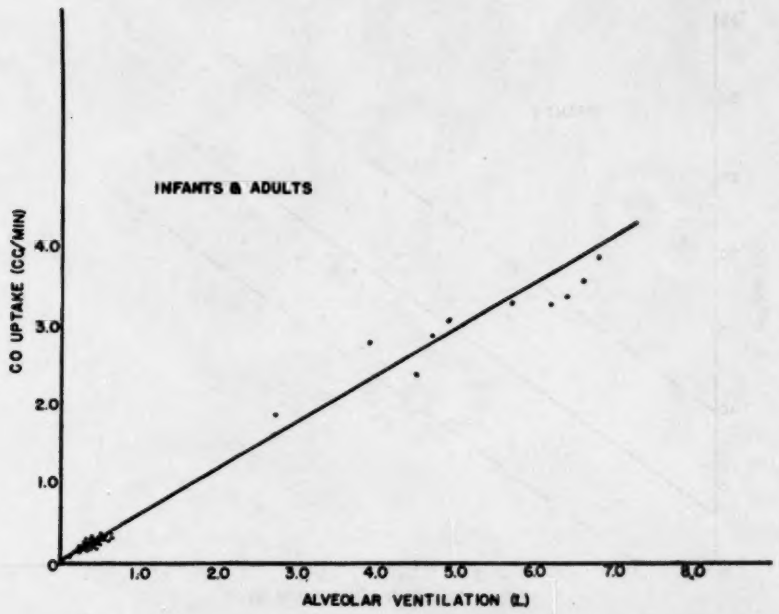

FIG. 3. THE CORRELATION OF CO UPTAKE IN ML. PER MINUTE WITH ALVEOLAR VENTILATION IN LITERS PER MINUTE IN 31 NORMAL NEWBORN INFANTS AS COMPARED WITH 12 NORMAL OLDER CHILDREN AND ADULTS

The solid line represents the regression line for the combined data.

babies and adults, and all were interpreted by the radiologists as normal. The X-rays of the babies were made within four hours after their diffusion studies.

Subjects

Ventilation and diffusion studies have been completed in 31 normal, newborn infants ranging in age from 9 to 96 hours, with a mean age of 38 hours. They varied in weight from 4 to 9 pounds, with a mean weight of 7.16 pounds. The babies were selected at random from a small staff newborn service. There were 18 males and 13 females. Ventilatory studies, carbon monoxide uptake and blood gases were measured on an additional number of babies on whom, for various technical reasons, diffusion measurements could not be completed.

For purposes of comparison, the same ventilation and diffusion studies were carried out on 12 normal older children and adults of varying body size.

Calculations

All definitions and symbols are those designated by the Committee on Standardization of definitions and symbols in respiratory physiology of the American Physiological Society (10). The minute ventilation, M_V, and alveolar ventilation, $\dot{V}_A$, were expressed in liters per minute at BTPS. The CO uptake in ml. of CO per minute was calculated as $\dot{V}_E\left(F_{Ico}\left(\frac{F_{EN_2}}{F_{IN_2}}\right) - F_{Eco}\right)$, where $\dot{V}_E$ is the total gas volume STPD expired per minute, F_I and F_E are gas concentrations in inspired and expired volumes, and the subscripts refer to the gases in question. The alveolar carbon monoxide tension was calculated by using a form of the Bohr relation for the alveolar equation and assuming that the alveolar CO_2 tension, P_ACO_2, is equal to the arterial CO_2 tension, P_aCO_2. The equation used was (13):

$$P_{Aco} = \frac{F_{Eco} - r\,F_{Ico}}{1 - r}\,(P_B - 47)$$

where

$$r = \frac{V_D}{V_T} = \frac{P_aCO_2 - P_ECO_2}{P_aCO_2}$$

RESULTS

It was felt that the "steady-state" was achieved in 31 of these babies, covering the time between the taking of femoral arterial blood and the collection of expired gas. In an additional 8 infants, although all data necessary for the calculation of D_{co} were obtained, the values for dead space to tidal volume ratios were found to be so abnormally high or low that the diffusion capacity

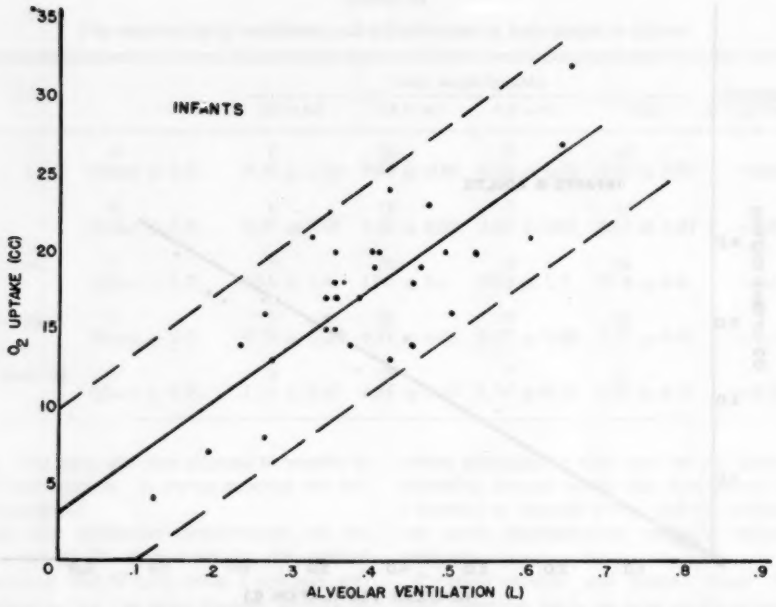

FIG. 4. THE CORRELATION OF OXYGEN UPTAKE IN ML. PER MINUTE WITH ALVEOLAR VENTILATION IN LITERS PER MINUTE IN 31 NORMAL NEWBORN INFANTS

The solid line represents the regression line for the data and the broken line represents two standard deviations around the regression line.

could not be calculated. It must be presumed that these babies were not in a steady state throughout the collection of blood and gas samples. Table I shows both direct determinations and data used in calculating the diffusion capacity on all infants. Table II shows both direct and calculated ventilatory data on all subjects.

The relationship of values obtained on the infants to body weight is shown in Table III.

The correlations of carbon monoxide uptake and oxygen uptake with alveolar ventilation in infants, and in infants combined with adults are shown in Figures 2, 3, 4, and 5. The correlation between oxygen uptake and carbon monoxide uptake in infants, and in infants combined with adults is shown in Figures 6 and 7.

DISCUSSION

The first question which will be reasonably brought up by pediatricians concerning such a study on newborn infants is the question of the safety of using carbon monoxide as a diffusion gas to be measured. If one takes the mean carbon monoxide uptake values for these babies of 0.23 ml. per min. with a mean weight of 7.16 pounds, and a mean total hemoglobin of 35 grams for babies of the same weight (19), and calculates the amount of hemoglobin combined with carbon monoxide in six minutes of breathing carbon monoxide at this low concentration, it amounts to approximately 1/35 of the baby's total hemoglobin. Even in conditions of respiratory distress, when all the hemoglobin may be needed for oxyhemoglobin combination, this would seem to be a negligible amount and would be the equivalent to the temporary removal of about 7 ml. of blood from a newborn infant.

The body plethysmograph which has been used previously in ventilatory studies in infants offers numerous disadvantages in studies requiring accurate measurements of gas uptake. In addition, the sensitivity of such a system is such as to dampen out the very small tidal volumes which occur with the rapid phase of small infants' arrhythmical breathing. The requirements of a system utilizing direct measurements of ventila-

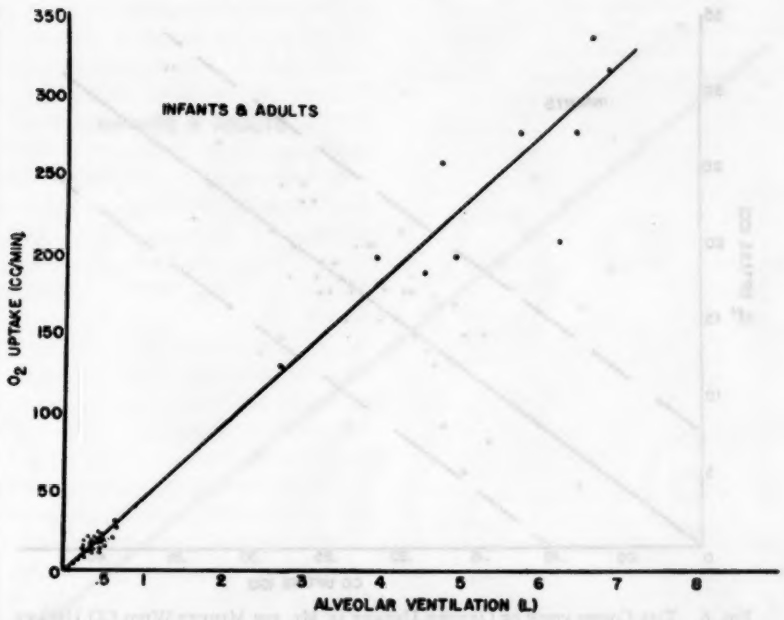

FIG. 5. THE CORRELATION OF OXYGEN UPTAKE IN ML. PER MINUTE AND ALVEOLAR VENTILATION IN LITERS PER MINUTE IN 31 NORMAL NEWBORNS AS COMPARED WITH 12 NORMAL OLDER CHILDREN AND ADULTS

The solid line represents the regression line for the combined data.

tion and gas uptake in a one-way circuit in small infants are primarily those of negligible resistance and dead space. Resistance to expiration was kept low by using the lowest resistance type of one-way valves, small "J" valves, the diameter of which was chosen as 5 mm. The tracheas of six infants dying between birth and six months of age were measured in their smallest diameter just above the coryna at autopsy and none was found to have dimensions greater than 4 by 6 mm. in their AP and lateral measurements. The use of a double "bag-in-box" system with a weight-compensated, low resistance spirometer interposed between the boxes contributed toward keeping the resistance to expiration minimal.

The anatomical dead space of the mask obviously varied slightly with how tightly the mask fitted the anatomical outlines of each baby's face. That the physiological dead space of the mask was quite small is demonstrated by the low physiological dead space measurements which were obtained on most babies, all of which could be accounted for by the upper respiratory passages.

The ventilatory measurements of minute ventilation and alveolar ventilation obtained in these infants agree favorably with those measurements found in the literature made indirectly with the body plethysmograph (1–9). Respiratory frequency in this series of babies is higher than in most series counted by other methods, and therefore the average tidal volumes are somewhat less than those in the literature. However, the dead space-tidal volume ratios are comparable to other series of normal newborns and therefore the physiological dead space measurements are less than those quoted. All these differences can be accounted for by the higher respiratory frequency recorded by this system. It should be noted, however, that this is a skewed distribution. There are several possible explanations for such increases in frequency. The infant's normal respiratory pattern might be disturbed by the mask and valve system in such a way as to increase the rate of breathing. Since minute ventilation, alveolar ventilation, carbon dioxide output, oxygen uptake and their ratios were undisturbed by the system, it is

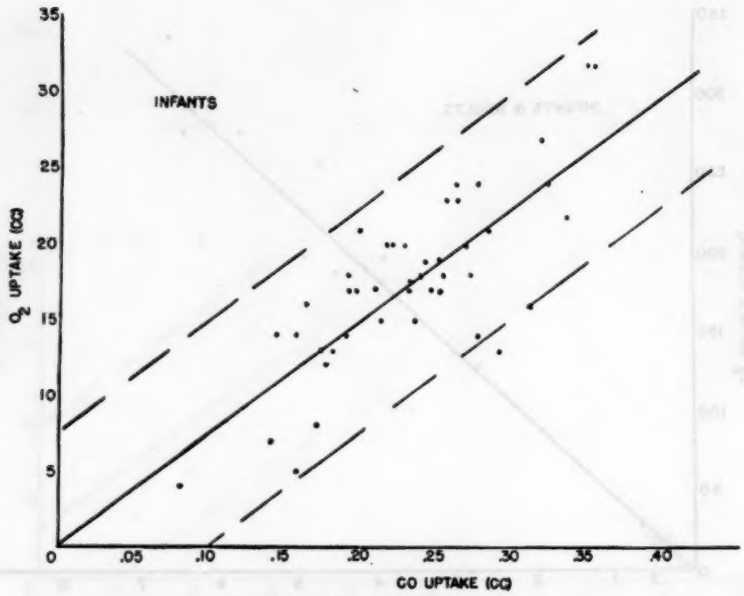

FIG. 6. THE CORRELATION OF OXYGEN UPTAKE IN ML. PER MINUTE WITH CO UPTAKE IN ML. PER MINUTE IN 31 NORMAL NEWBORN INFANTS

The solid line represents the regression line for the data and the broken line represents two standard deviations around the regression line.

unlikely that this increased rate was the result of any significant change in the overall pattern of the infants' total respiratory physiology. Other possible factors to account for this difference in rate are the sensitivity of this system to small fluctuations in respiratory pattern as compared with that of the body plethysmograph, and the possible difference in ambient temperature between laboratories. The usual temperature of the room in which these measurements were made was between 85° and 90° F. In addition, the baby was swaddled in a sheet or light blanket restraint. Additional evidence that the increased rate was not due to a disturbance of the normal resting pulmonary physiology and metabolism is that the ventilatory values, when plotted against the gas uptake values, are well correlated in both infants and adults despite the fact that they were measured by different techniques as far as apparatus dead space and resistance are concerned.

Another factor in judging the validity of these data is the assumption of the steady-state from the time of doing the femoral arterial puncture through the end of the gas collection. If any significant crying occurred at any time the procedure was abandoned from that point. To test the assumption that the arterial partial pressure of carbon dioxide, as determined from blood taken from ten to fifteen minutes before the gas collection, represented the alveolar partial pressure of carbon dioxide during the time of gas collection, the Henderson continuous alveolar sampler was modified for infants (20). A series of 18 consecutive determinations was made comparing arterial partial pressure of carbon dioxide, as calculated from arterial whole blood carbon dioxide content and arterial pH, and the alveolar partial pressure of carbon dioxide obtained directly from the alveolar sample taken during the subsequent fifteen minutes. These data are shown in Table IV. Two of the differences were so large that gross error in determinations or an unsteady state must have occurred. For the 16 other pairs, the air determinations exceeded the blood determinations by 0.2 mm. Hg on the average, with a standard error of $\pm$ 0.4 mm. Hg, indicating no evidence of

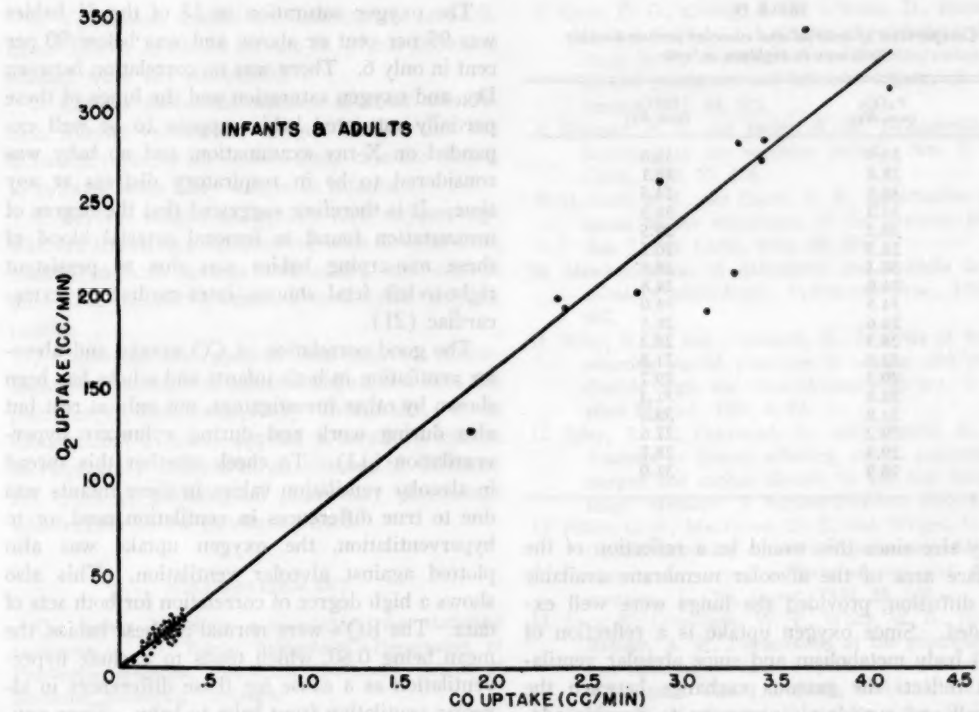

FIG. 7. THE CORRELATION OF OXYGEN UPTAKE IN ML. PER MINUTE WITH CO UPTAKE IN ML. PER MINUTE IN 31 NORMAL NEWBORN INFANTS AS COMPARED WITH 12 NORMAL OLDER CHILDREN AND ADULTS

The solid line represents the regression line for the combined data.

bias. The standard deviation for the distribution of differences of the 16 pairs was 1.5 mm. Hg. These data lend validity to the assumption that the steady-state was usually achieved, but also show that occasionally when this is not the case the calculated values for partial pressure of carbon dioxide might be a source of error of considerable magnitude. As has been mentioned, this was presumably true in 8 of 39 babies.

The validity of calculating the arterial partial pressure of carbon dioxide from arterial pH measured at room temperature and the whole blood carbon dioxide content was checked by placing six 10-ml. samples of whole blood in a 500-ml. tonometer, filling the tonometer with a known concentration of carbon dioxide (about 5 per cent), incubating it with constant rotation at 37° C. for 45 minutes and analyzing the blood for pH, oxygen and carbon dioxide content and oxygen capacity as usual. The arterial partial pressure of carbon dioxide was then calculated and compared with the known carbon dioxide tension with a mean difference of 1.03 mm. Hg and a greatest difference of 1.63 mm. Hg, and it was assumed that the usual method of calculating P_aCO_2 was satisfactorily accurate.

The sources of error inherent in the measurement of pulmonary gaseous diffusion using the steady-state carbon monoxide method have been discussed in detail by Filley, MacIntosh, and Wright (13).

The factors which might be expected to modify the diffusion capacity of gas, assuming that the measurements are accurate, are the total surface area of the alveolar membrane in contact with patent capillaries, the thickness of this membrane and its permeability. As yet these three properties of the membrane cannot be adequately separated. It might be expected that carbon monoxide uptake would have a positive correlation with

TABLE IV

Comparison of arterial and alveolar carbon dioxide tensions in eighteen infants

P_ACO_2 (*mm. Hg*)	P_aCO_2 (*mm. Hg*)
33.9	36.6
28.5	28.1
34.1	24.3
33.3	31.3
30.2	29.5
28.5	26.5
30.4	28.8
24.6	24.8
34.5	34.0
24.6	26.5
28.3	28.2
32.8	31.8
29.5	29.7
24.8	31.3
31.8	33.3
29.2	27.6
29.9	28.7
28.9	31.0

body size since this would be a reflection of the surface area of the alveolar membrane available for diffusion, provided the lungs were well expanded. Since oxygen uptake is a reflection of total body metabolism and since alveolar ventilation reflects the gaseous exchange between the alveoli and outside air necessary to provide adequate oxygen uptake and carbon dioxide output, it would be expected that alveolar ventilation and oxygen uptake would show positive correlations with body weight as is the case. The alveolar partial pressure of carbon monoxide, however, would not be expected to vary with body size but with the dead space to tidal volume ratio, alveolar ventilation and D_{CO} and no correlation can be shown between P_ACO and body weight. Since D_{CO} is a calculated ratio between CO uptake and P_ACO, it is understandable that, with small differences in body size among the babies studied, the differences in D_{CO} from one weight group to another are not statistically different although there is a progressive increase in D_{CO} demonstrated in each increase in weight group among the infants.

It can be noted that the pH of arterial blood in these babies was within the normal adult range and that the arterial plasma CO_2 content was considerably lower. This made the P_aCO_2 in the newborn as calculated from the Henderson-Hasselbach equation lower than the normal adult level by an average of 8 to 10 mm. Hg.

The oxygen saturation in 15 of the 31 babies was 95 per cent or above, and was below 90 per cent in only 6. There was no correlation between D_{CO} and oxygen saturation and the lungs of these partially saturated babies appear to be well expanded on X-ray examination, and no baby was considered to be in respiratory distress at any time. It is therefore suggested that the degree of unsaturation found in femoral arterial blood of these non-crying babies was due to persistent right-to-left fetal shunts, intra-cardiac or extra-cardiac (21).

The good correlation of CO uptake and alveolar ventilation in both infants and adults has been shown by other investigators, not only at rest but also during work and during voluntary hyperventilation (13). To check whether this spread in alveolar ventilation values in these infants was due to true differences in ventilation need, or to hyperventilation, the oxygen uptake was also plotted against alveolar ventilation. This also shows a high degree of correlation for both sets of data. The RQ's were normal in these babies, the mean being 0.80, which tends to exclude hyperventilation as a cause for these differences in alveolar ventilation from baby to baby. Since oxygen consumption should not be affected in the same way as CO uptake by hyperventilation, the data for oxygen uptake and CO uptake were plotted against each other for both infants and adults. They correlate well, with the origins of the regression lines passing through zero. This is further evidence that hyperventilation was not an important factor in values obtained for D_{CO} in these infants.

SUMMARY AND CONCLUSIONS

1. The Filley steady-state carbon monoxide method of estimating the diffusion of gases across the pulmonary alveolar-capillary membrane has been modified for use in newborn infants.

2. A method for making direct ventilation and gas uptake measurements utilizing a face mask and double Donald-Christie box with a spirometer interposed between the boxes has been designed for use in infants.

3. Diffusion and ventilation studies have been carried out in 31 normal newborn infants and in 12 normal older children and adults for comparison.

4. Ventilation studies are described in an additional fifteen newborn infants in whom diffusion studies were not completed.

5. Carbon monoxide uptake, alveolar ventilation, minute ventilation, oxygen uptake, and the diffusion capacity for carbon monoxide are shown to increase with body weight in infants.

6. Carbon monoxide uptake and oxygen uptake are shown to correlate well with alveolar ventilation in both infants and adults and carbon monoxide uptake correlates well with oxygen uptake.

7. Although the method has inherent drawbacks as a measure of gaseous diffusion across the pulmonary alveolar-capillary membrane, it is thought that it can be applied to newborn infants in respiratory distress in an attempt to delineate the types of pathological pulmonary physiology which occur in the newborn period.

ACKNOWLEDGMENT

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TYPE-SPECIFIC STREPTOCOCCAL ANTIBODY [1]

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Immunity to group A streptococcal infections has been shown to be associated with the formation of antibody to the type-specific component of the streptococcus, M-protein (1). In spite of its apparent importance, little is known concerning this antibody. A variety of techniques has been employed in the study of type-specific antibody but for practical purposes the bacteriostatic test is the only method which has proved to be satisfactory. Using this technique, Kuttner and Lenert (2) reported that, following infection with a Type 36 streptococcus, all patients developed type-specific antibody, and that the antibody persisted for long periods of time and was unrelated to the development of antistreptolysin O. These authors, however, were unable to demonstrate significant type-specific antibody in patients following infection with Types 15 and 19 streptococci. In extensive studies of the bacteriostatic test in three patients, Rothbard (3) found that type-specific antibody appeared 3 to 5 weeks following infection and persisted for at least 37 weeks. Rothbard, Watson, Swift, and Wilson (4) observed that bacteriostatic antibody appeared in 76 per cent of 82 streptococcal infections (71 patients) 2 to 10 weeks following onset of the disease; patients who developed rheumatic fever exhibited rises more frequently than did those patients who did not suffer this complication. In addition, these authors reported that bacteriostatic antibody appeared later than antistreptolysin O and that the rise in bacteriostatic antibody was slightly delayed in patients who developed rheumatic fever. In a study of patients with scarlet fever treated with penicillin, Daikos and Weinstein (5) reported that the administration of penicillin suppressed formation of type-specific bacteriostatic antibody and that there was a relation between the dose and route of administration of penicillin and the degree of suppression.

In the studies to be described, the time of development of type-specific bacteriostatic antibody in patients with untreated infections due to several types of group A streptococcus was determined; the effect of penicillin and Aureomycin® treatment on the inhibition of type-specific antibody as related to the persistence of the streptococcus was studied; and the time of development of type-specific antibody in patients with rheumatic fever and uncomplicated streptococcal infections was compared. In most instances, the development of this antibody was compared with the development of the more widely studied streptococcal antibody, antistreptolysin O.

DESCRIPTION OF STUDY

This study was conducted at Francis E. Warren Air Force Base, Wyoming, on airmen whose ages were between 19 and 21 years. The following criteria for inclusion in the study were employed: 1) The presence of exudative pharyngitis or tonsillitis, 2) a peripheral leucocyte count of 10,000 or greater, 3) the isolation of typable group A streptococci from the throat culture, and 4) availability for follow-up examinations for at least three months after the acute infection. In addition, a small group of patients with streptococcal infections who did not meet all the above criteria but who subsequently developed acute rheumatic fever was included.

Ninety-four patients fulfilled the above criteria and were admitted to the study. Forty-nine patients had uncomplicated streptococcal infections and were given no specific treatment; classification of the streptococci isolated from the throat cultures of these patients showed that 9 were Type 5, 9 were Type 6, 12 were Type 24, and 19 were Type 14. Thirteen patients with rheumatic fever were included; 8 had previously had Type 14 infec-

[1] This investigation was conducted under the sponsorship of the Commission on Acute Respiratory Diseases and the Commission on Streptococcal Diseases, Armed Forces Epidemiological Board, and was supported by the Offices of The Surgeons General, Departments of the Army and Air Force, Washington, D. C.

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tions and 5 had had Type 24 infections. Twenty-two patients with Type 14 streptococcal infections were treated with Aureomycin®, and 10 patients with Type 14 streptococcal infections were treated with penicillin. These patients all had uncomplicated infections and were treated at random as part of another study. Aureomycin® therapy consisted of the oral administration of a total dose of 10.5 Gm. over a period of five days. Procaine penicillin G in sesame oil with 2 per cent aluminum monostearate was administered by intramuscular injection in doses of 600,000 units on admission to the study, and again in 72 hours.

Throat cultures and sera were obtained from all patients at the time of admission to the hospital, at approximately 2-week intervals for 2 months, and then at monthly intervals. Cultures were taken with dry sterile cotton swabs and streaked directly onto sheep-blood agar. In most instances another swab was also incubated in Pike's selective broth (6) for 18 hours and then subcultured on sheep-blood agar. Beta-hemolytic streptococci were classified according to the method of Swift, Wilson, and Lancefield (7). Antistreptolysin O titers of all sera from each patient were determined concomitantly by a modification of the technique of Hodge and Swift (8). Differences of two dilution increments (0.2 log) were considered indicative of infection.

Type-specific antibody was measured by a modification of the technique as reported by Rothbard (3). Details of this method are as follows:

Streptococcal strains. Strains of streptococci freshly isolated from throat cultures of patients acutely ill with streptococcal infections were used in all experiments.

Preparation of cultures. Stock strains of streptococci were kept at −20° C. These were prepared by growing the streptococcus in fresh beef-heart infusion broth for 6 to 8 hours, adding 2 per cent sterile rabbit or sheep serum, and rapidly freezing in small aliquots in a carbon dioxide ice-alcohol bath. The day before each experiment, 0.25 cc. of the stock culture was added to 5 cc. of beef-heart infusion broth to which had been added a drop of sheep blood. This culture was incubated for eight hours at 37° C. with frequent shaking. One-tenth cubic centimeter of this rapidly growing culture was added to 5 cc. of beef-heart infusion broth and incubated at 37° C. for 15 hours. Tenfold serial dilutions of this culture were made in dextrose phosphate broth and were the dilutions used in the bacteriostatic test. The number of chains of streptococci per cubic centimeter was calculated by pouring sheep blood agar plates containing 0.5 cc. of the 10^{-6} dilution and 1.0 cc. of the 10^{-7} dilution. The 10^{-6} dilution usually contained 100 to 200 colonies per cc., and the 10^{-7} dilution, 10 to 20 colonies per cc.

Source of blood. Blood from normal adults which showed no bacteriostatic activity for the streptococci under investigation was used in all experiments. Heparin in a final dilution of 1:30,000 was used as an anticoagulant. Leucocyte counts were performed on all specimens and were always above 4500 per cubic millimeter.

Bacteriostatic test. All sera from each patient were tested concomitantly against homologous and heterologous types of streptococci. Five-hundredths of a cubic centimeter of each serum was added to a series of six tubes (100 by 7 mm.). To these tubes was added 0.05 cc. of the appropriate dilution of culture, 10^{-4} through 10^{-6}. Freshly drawn heparinized blood, 0.25 cc., was then added to each tube. The tubes were sealed in an oxygen-gas flame and incubated at 37° C., in an electrically driven machine which rotated the tubes on the long axis at 8 R.P.M. Gross readings for the presence of hemolysis of the blood were made after 14 hours; the presence of hemolysis indicated growth of the streptococcus and absence of bacteriostatic antibody; the absence of hemolysis indicated failure of the streptococcus to grow and the presence of bacteriostatic antibody. Differences in readings of 2 tubes (2 logs) or greater were considered significant.

TABLE I

The development of type-specific antibody and antistreptolysin O in patients with untreated infections due to different types of Group A streptococci

Streptococcus type	No. cases	Patients developing significant antibody increase*			
		Type-specific antibody		Antistreptolysin	
		No.	Per cent	No.	Per cent
5	9	8	89	9	100
6	9	9	100	9	100
14	19	17	89	19	100
24	12	11	92	11	92
Total	49	45	92	48	98

* Type-specific antibody—2 logs or greater; antistreptolysin O—0.2 log or greater.

RESULTS

The development of type-specific antibody and antistreptolysin O in patients with untreated and uncomplicated infections due to Types 5, 6, 14 and 24 group A streptococci is shown in Table I. Type-specific antibody developed in 45 of 49 patients, or 92 per cent, while a diagnostic increase in antistreptolysin was demonstrated in 48, or 98 per cent, of the patients. The difference in the percentage of patients who developed these two antibodies according to the type of the infecting streptococcus was insignificant.

Figure 1 shows the time of development of antistreptolysin O in the same group of patients. This figure shows, by 10-day periods following the onset of the streptococcal infection, the cumulative per cent of patients who developed a 2-tube or significant rise of antistreptolysin. By the end of 30 days the majority of patients had developed in-

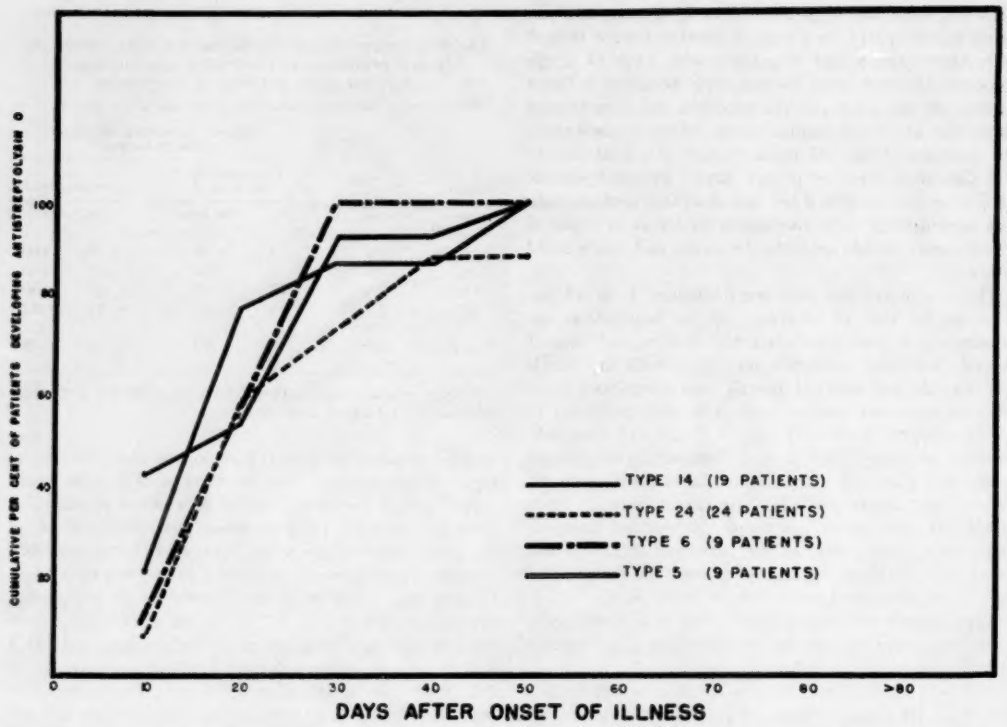

FIG. 1. TIME OF ANTISTREPTOLYSIN O RESPONSE IN PATIENTS WITH INFECTIONS DUE TO DIFFERENT TYPES OF GROUP A STREPTOCOCCI

Serum samples were not obtained from all individuals in every 10-day period in the studies presented in Figures 1 through 4. The values for each period, however, were calculated using the total number of individuals in the group as the denominator; patients were included in the numerator at the time of the demonstrated antibody increase, regardless of the time that had elapsed since the previous antibody determination. This was considered justifiable after it had been demonstrated that the shapes and relative positions of the curves were almost identical when patients were included in the numerator in the period immediately following the time of the last serum that showed no antibody increase, regardless of the time that elapsed until the antibody increase was exhibited.

creases in antistreptolysin and there was no significant difference among patients with infections due to different types of streptococci.

The time of development of type-specific antibody in the same patients is shown in Figure 2. Those patients with Type 24 infections developed antibody most rapidly, so that by 40 days after the onset of the acute illness 83 per cent of patients had shown significant rises. This is in contrast to the development of type-specific antibody in those patients with Type 5 infections. By 40 days only 11 per cent had developed antibody, and it was not until after 80 days that 88 per cent had shown significant rises. Patients with Type 6 and Type 14 infections developed type-specific antibody at rates that were between these two extremes. It is apparent that antistreptolysin O developed rapidly following streptococcal infections and the time of development was not altered by the infecting type of streptococcus. In contrast, type-specific antibody developed slowly and the rate at which this antibody appeared was dependent on the serological type of the infecting organism.

The time of development of type-specific antibody in the eight patients with Type 14 infections, and in five patients with Type 24 infections who subsequently developed rheumatic fever, was the

same as in the patients with uncomplicated infections due to these same types.

Figure 3 shows the development of antistreptolysin in patients with Type 14 streptococcal infections as it is affected by treatment with Aureomycin® or penicillin. The group of patients who received no treatment is the same as that shown in Figures 1 and 2. Aureomycin® therapy caused only slight inhibition to the development of antistreptolysin, while penicillin therapy resulted in marked suppression. By 30 days only 40 per cent of patients treated with penicillin had shown a significant increase in titer, and it was not until 60 days that 80 per cent of the patients had a 2-tube rise.

Figure 4 shows the development of type-specific antibody in the same group of patients. Aureomycin® treatment resulted in partial inhibition of this antibody; only one patient had developed antibody before 50 days, and only a little over 60 per cent of patients developed antibody by the time the study was terminated. In contrast to this, no patients in the penicillin treated group developed type-specific antibodies, even though they were followed for periods of up to 115 days.

Because of the marked differences that were seen between the group treated with Aureomycin® and that treated with penicillin in the development of both kinds of antibody, an attempt was made to explain these differences. The effect of treatment on the persistence of the infecting type of streptococcus in the follow-up cultures is shown in Table II. All patients who received no therapy continued to harbor the infecting type of streptococcus; of the 10 patients treated with penicillin, 1 patient had one positive culture following treatment; of the 22 patients treated with Aureomycin®, 19 showed a positive culture at some time

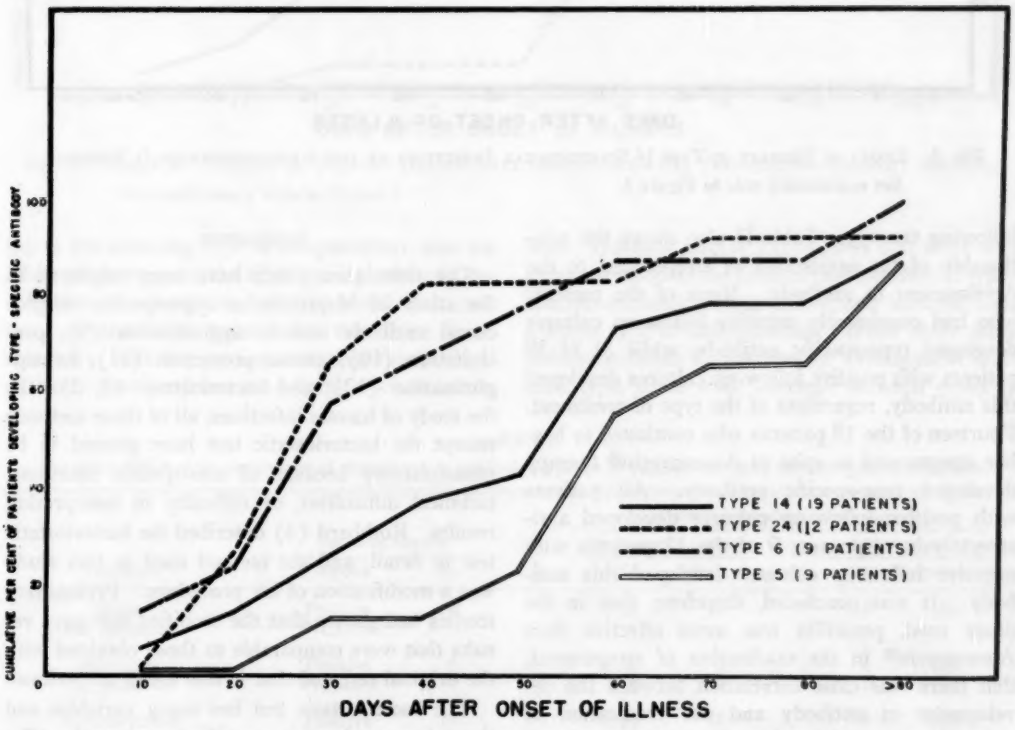

FIG. 2. TIME OF DEVELOPMENT OF TYPE-SPECIFIC ANTIBODY IN PATIENTS WITH INFECTIONS DUE TO DIFFERENT TYPES OF GROUP A STREPTOCOCCI

See explanatory note to Figure 1.

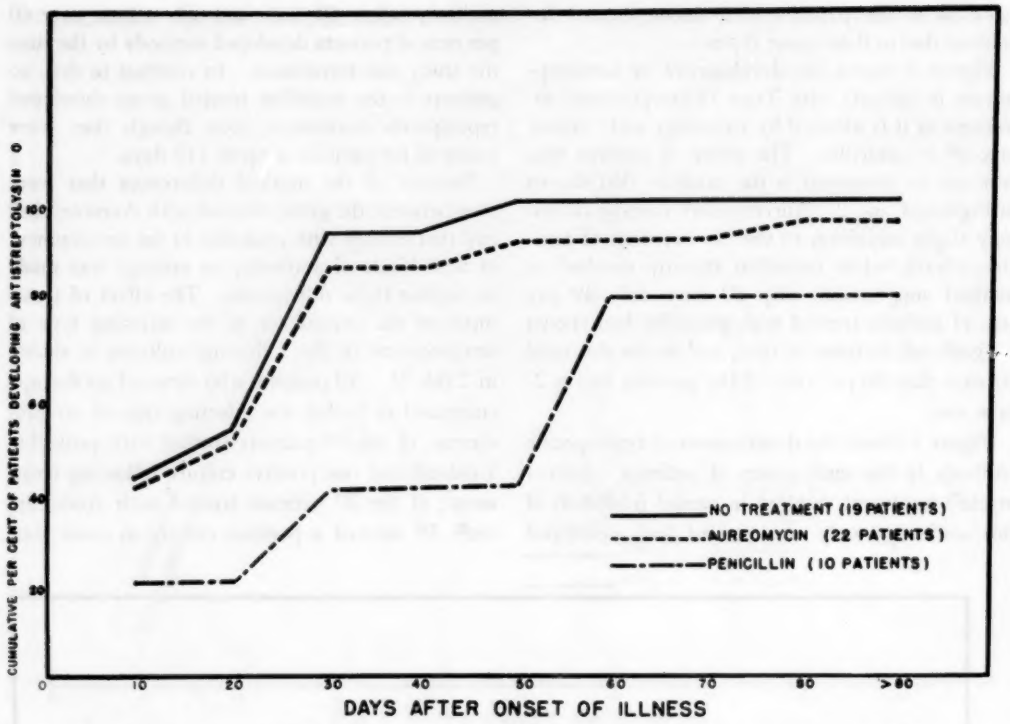

FIG. 3. EFFECT OF THERAPY OF TYPE 14 STREPTOCOCCAL INFECTIONS ON THE ANTISTREPTOLYSIN O RESPONSE
See explanatory note to Figure 1.

following therapy. Table II also shows the relationship of the persistence of streptococci to the development of antibody. None of the patients who had consistently negative follow-up cultures developed type-specific antibody, while 31 of 39 patients with positive follow-up cultures developed this antibody, regardless of the type of treatment. Fourteen of the 19 patients who continued to harbor streptococci in spite of Aureomycin® therapy developed type-specific antibody. All patients with positive follow-up cultures developed antistreptolysin responses; 9 of the 12 patients with negative follow-up cultures developed this antibody. It was concluded, therefore, that in the doses used, penicillin was more effective than Aureomycin® in the eradication of streptococci, that there was close correlation between the development of antibody and the eradication of streptococci, and that this effect was more pronounced in the case of type-specific antibody than in the case of antistreptolysin.

DISCUSSION

The techniques which have been employed in the study of M-protein or type-specific streptococcal antibody include agglutination (9), precipitation (10), mouse protection (11), hemagglutination (12), and bacteriostasis (2, 3). In the study of human infections, all of these methods except the bacteriostatic test have proved to be unsatisfactory because of non-specific reactions, technical difficulties, or difficulty in interpreting results. Rothbard (3) described the bacteriostatic test in detail, and the method used in this study was a modification of his procedure. Preliminary studies had shown that the modified test gave results that were comparable to those obtained with the original test and that it was easier to perform.

The bacteriostatic test has many variables and there is no adequate quantitative control. The differences shown in the present paper in the time of development of type-specific antibody accord-

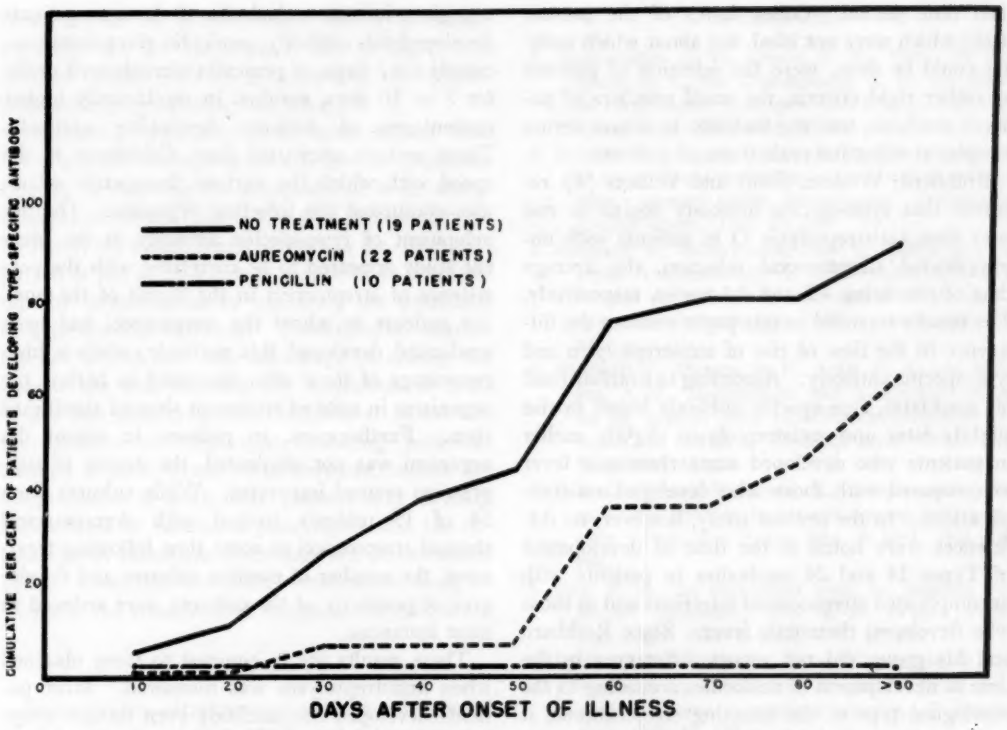

FIG. 4. EFFECT OF THERAPY OF TYPE 14 STREPTOCOCCAL INFECTIONS ON THE TYPE-SPECIFIC ANTIBODY RESPONSE
See explanatory note to Figure 1.

ing to the infecting type of streptococcus, and the effect of therapy on the type-specific antibody response must, therefore, be interpreted with caution. It should be pointed out that there is no assurance, with the present test, that the levels of antibody measured are the effective levels in the host. It should also be emphasized that there was great variation in the time of development of type-specific antibody among individuals infected with a single type of streptococcus. While there were definite patterns in the time of development with each type, there were overlaps between types at

TABLE II

The relationship of the persistence of streptococci to antibody response in patients receiving various types of treatment

Treatment	Follow-up cultures		Patients developing antibody increase			
			Type-specific antibody		Antistreptolysin	
	Results	No.	No.	Per cent	No.	Per cent
None (19 patients)	Positive	19	17	89	19	100
	Negative	0				
Penicillin (10 patients)	Positive	1	0	0	1	100
	Negative	9	0	0	7	78
Aureomycin® (22 patients)	Positive	19	14	74	19	100
	Negative	3	0	0	2	67
Total	Positive	39	31	79	39	100
	Negative	12	0	0	9	75

each time period. Other facets of the present study which were not ideal, but about which nothing could be done, were the selection of patients by rather rigid criteria, the small numbers of patients available, and the inability to obtain serum samples at exact intervals from all patients.

Rothbard, Watson, Swift and Wilson (4) reported that type-specific antibody begins to rise later than antistreptolysin O in patients with uncomplicated streptococcal infection, the average time of rise being 4.2 and 2.4 weeks, respectively. The results reported in this paper confirm the difference in the time of rise of antistreptolysin and type-specific antibody. According to Rothbard and his associates, type-specific antibody began to rise slightly later and antistreptolysin slightly earlier in patients who developed acute rheumatic fever as compared with those who developed no complications. In the present study, however, no differences were noted in the time of development of Types 14 and 24 antibodies in patients with uncomplicated streptococcal infections and in those who developed rheumatic fever. Since Rothbard and his group did not report differences in the time of development of antibodies according to the serological type of the infecting streptococcus, it is possible that the difference they observed might have been due to a difference in distribution of infecting types between the uncomplicated group and the group developing rheumatic fever. It is also possible that the number of patients observed, particularly in the present study, was too small to demonstrate significant differences of this type.

Stetson (13) and Rantz, Boisvert, and Clark (14), have reported that antistreptolysin O response varies with the infecting type of streptococcus. Rantz and his group found differences in the magnitude and time of response, while Stetson reported only on the magnitude of the responses. It is possible that some of the observed differences in the time of the response may be significant, but the present data do not allow this conclusion. In contrast, the differences in the time of development of type-specific antibody according to the serological type in these same patients are quite marked.

Daikos and Weinstein (5), using Rothbard's technique, reported that penicillin treatment of streptococcal infections suppresses the development of type-specific antibody. When penicillin was given intramuscularly for 10 days, no patients developed this antibody; penicillin given intramuscularly for 7 days, or penicillin administered orally for 7 or 10 days, resulted in significantly higher percentages of patients developing antibody. These authors attributed these differences to the speed with which the various therapeutic schedules eliminated the infecting organism. The development of type-specific antibody in the present study appeared to be correlated with the persistence of streptococci in the throat of the host. No patients in whom the streptococci had been eradicated developed this antibody, while a high percentage of those who continued to harbor the organisms in spite of treatment showed significant rises. Furthermore, in patients in whom the organism was not eliminated, the degree of suppression seemed important. While cultures from 14 of 19 patients treated with Aureomycin® showed streptococci at some time following treatment, the number of positive cultures and the degree of positivity of the cultures were reduced in most instances.

These results are in contrast to those obtained when antistreptolysin was measured. Most patients developed this antibody even though streptococci were eradicated. It is of note that in the penicillin-treated group 80 per cent of patients eventually developed significant increases of this antibody, yet in four patients the increase was not observed until 50 days following the onset of the infection. This failure of penicillin to prevent completely the formation of antistreptolysin, in spite of the apparent eradication of the streptococcus and the complete inhibition of type-specific antibody, suggests that the major antigenic stimulus to the formation of antistreptolysin occurs early in the course of streptococcal infections, while the stimulus to the formation of type-specific antibody occurs over a much longer period. This observation is in agreement with data reported by Brock and Siegel (15). These authors found that treatment of streptococcal pharyngitis with penicillin within 31 hours after the onset of the disease was more effective in the suppression of antistreptolysin formation than therapy delayed for 3 or 5 days. It is also possible that the more frequent antistreptolysin response represents a recall phenomenon, in that the patients probably had had previous contact with streptolysin and a

small amount of antigen might induce detectable antibody; on the other hand, they probably had not had contact with the M-protein of the streptococcus causing the observed infection.

Previously published data have shown that Aureomycin® therapy is as effective as penicillin therapy in relieving the symptoms of acute streptococcal infections (16). The data presented here, however, show that Aureomycin® in the doses employed is not as effective as penicillin in eradicating the streptococcus or inhibiting the formation of type-specific antibody. Theoretically, Aureomycin® would have the advantage of curing the patient but still allowing the formation of antibodies that protect against reinfection. However, the chances of reinfection following penicillin therapy are not great. Furthermore, unless the streptococcus is eradicated, patients may develop recurrences of exudative pharyngitis and the attack rate of rheumatic fever may not be decreased (17). Penicillin, therefore, remains the drug of choice in the treatment of streptococcal infections.

SUMMARY

Type-specific streptococcal antibody developed in the majority of individuals following streptococcal respiratory infections. In contrast to antistreptolysin, this antibody developed slowly and showed marked variation in the time of development according to the serological type of the infecting streptococcus. Treatment with penicillin or Aureomycin® inhibited the formation of this antibody, penicillin causing the greater degree of inhibition. The degree of inhibition appeared to be related to the successful elimination of the organism by therapy.

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HYPOXEMIA AND CORONARY BLOOD FLOW [1, 2]

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Hypoxemia has long been known to produce a decrease in resistance in the vascular system of the heart. From studies on the effect of hypoxemia on coronary blood flow in the dog heart-lung preparation, Hilton and Eichholtz (2) reached the conclusion that the decrease in coronary resistance produced by hypoxemia is due to a direct action of low arterial oxygen tension on the smooth muscle of the vessels. However, the evidence in support of this contention is indirect and does not exclude myocardial hypoxia as the initiating factor.

If it can be demonstrated that under certain experimental conditions, which exclude myocardial hypoxia, a reduction in coronary arterial oxygen content does not increase coronary blood flow, then it would appear doubtful that low arterial oxygen tension has a direct action on the smooth muscle of the coronary vessels. This report is concerned with experiments in which high coronary perfusion pressures were employed in an attempt to determine the effect of low arterial oxygen tension on the coronary vessels in the absence of myocardial hypoxia.

METHODS

I. Experiments on the intact beating heart

Eleven technically satisfactory experiments were performed on open-chest dogs anesthetized with pentobarbital (30 mgm. per Kg.).

Preparation. The chest was opened in the fourth left intercostal space and artificial respiration started. Following administration of heparin [3] the left common coronary artery was cannulated with an Eckstein cannula (3) and perfused from a carotid artery via a pump perfusion system (Figure 1). By means of this perfusion system any desired perfusion pressure could be obtained regardless of aortic pressure. The coronary sinus was cannulated and the sinus outflow routed to the right jugular vein. Coronary blood flow was measured by an optically recording rotameter (4). Perfusion pressure was kept constant by means of a screw clamp and an air outlet at the top of the reservoir and was measured at a point just proximal to the coronary cannula. Perfusion and aortic pressures were optically recorded by modified Gregg manometers.

Deoxygenation of blood. Arterial blood from the experimental animal was deoxygenated by passing it directly from a femoral artery through the lungs of a recently sacrificed dog whose lungs were intermittently inflated with 5 per cent CO_2 in nitrogen. Different degrees of desaturation could be obtained by varying the rate of blood flow through the lungs, varying the respiratory cycle in the isolated lungs, or altering the oxygen content of the gas used to inflate the isolated lungs. For control periods, femoral arterial blood was passed through lungs intermittently inflated with 5 per cent CO_2 in oxygen.

Experimental procedure. The deoxygenated or oxygenated arterial blood was pumped through tube A (Figure 1) into the reservoir, and thence through the system into the left coronary artery at constant perfusion pressures. Records of pressure and flow and samples of coronary artery and coronary sinus blood were obtained two minutes after perfusion with deoxygenated or oxygenated blood was started. Coronary blood flow and coronary sinus oxygen content were stabilized at this time. Analyses for oxygen content were done in duplicate by the method of Roughton and Scholander (5). Between experimental periods perfusion of the left coronary artery was maintained by clamping the tubing at A and unclamping at B (Figure 1). Arterial pressure was kept nearly constant in the experimental animal by means of a Lampson bottle connected to one femoral artery. At no time was there systemic hypoxia since artificial respiration with room air was continued throughout the experiment. At the conclusion of each experiment the perfused part of the heart was stained with India ink, excised and weighed.

II. Fibrillating heart preparation

In two experiments, a dog fibrillating heart preparation was employed and the left coronary artery was perfused. The fact that the right coronary artery was not perfused does not affect the results in these experiments, since 1) flows were compared at equal perfusion pressures during control and hypoxemia periods, and 2) collateral flow between right and left coronary arteries is less than one cubic centimeter of blood per minute (6).

[1] Supported by a grant from the Cleveland Area Heart Society.

[2] Published previously as a preliminary report (1).

[3] Kindly supplied by the Upjohn Company, Kalamazoo, Michigan.

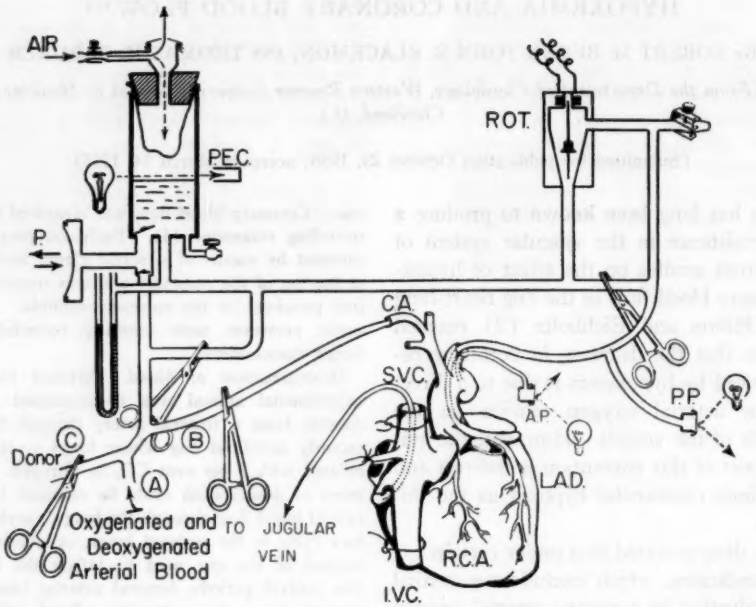

FIG. 1. DIAGRAM OF THE APPARATUS

P. = Modified Dale-Schuster pump; P.E.C. = Photoelectric cell with relay to start pump when level in reservoir falls; ROT. = Rotameter; C.A. = Carotid artery; S.V.C. = Superior vena cava; I.V.C. = Inferior vena cava; L.A.D. = Left anterior descending coronary artery; R.C.A. = Right coronary artery; P.P. = Perfusion pressure; A.P. = Aortic pressure.

The vena cavae and azygos vein were ligated and the main pulmonary artery was cannulated centrally. Between experimental periods blood from a donor dog perfused the left coronary artery via tube C (Figure 1) and was collected from the pulmonary artery and periodically returned to the donor. In these experiments the lungs of the experimental animal (fibrillating heart) were used for deoxygenating the donor dog's arterial blood in the same manner as for the experiments on the beating heart.

RESULTS

In the first two experiments (Table I), coronary artery perfusion pressure was set at 100 mm. Hg. In these experiments control coronary sinus oxygen was in the upper level of the normal range. However, when the coronary arterial oxygen content was reduced to approximately half the control values, the sinus oxygen content reached very low levels and coronary blood flow exhibited a large increase. Aortic pressure, heart rate, and myocardial oxygen consumption remained relatively constant.

In the third experiment, coronary artery perfusion pressure of 130 mm. Hg produced high coronary sinus oxygen levels when coronary arterial blood was fully saturated with oxygen. Reducing arterial oxygen content from 23.1 to 8.9 volumes per cent resulted in an increase in coronary flow and a reduction of coronary sinus oxygen content from 12.5 to 2.2 volumes per cent. It was only by virtue of the increment in blood flow that the myocardial oxygen supply was maintained. In periods 4 and 6 of Experiment 3 arterial oxygen content was reduced so drastically that the extraction of oxygen from blood perfusing the myocardium was almost complete. Despite the large increases in blood flow in periods 4 and 6 the cardiac oxygen supply was impaired and myocardial oxygen consumption fell below control levels.

In Experiments 4 and 5, control coronary sinus oxygen contents were within the normal range and the increments in coronary blood flow

TABLE I

Effect of reduction in arterial oxygen content on coronary blood flow at different perfusion pressures

Exp. No.	CBF	Perfusion pressure	Arterial O_2	Coronary sinus O_2	O_2 cons.	Heart rate	Aortic pressure
	cc./min./100 gm.	*mm. Hg*	*vol. %*	*vol. %*	*cc./min./100 gm.*	*beats/min.*	*mm. Hg*
1	80	98	21.1	9.2	9.5	180	119/98
	113	102	11.3	3.3	9.0	170	130/104
	84	96	19.1	7.8	9.5	170	120/96
2	55	102	21.1	6.4	8.1	156	122/108
	95	100	12.1	2.8	8.8	156	122/107
	62	97	21.3	8.0	8.3	156	122/108
	109	93	9.7	1.6	8.8	156	124/105
	62	104	22.1	8.3	8.6	156	126/110
	111	97	9.9	1.7	9.2	156	121/101
	74	102	20.6	8.5	9.0	156	119/105
3	65	133	23.1	12.5	6.9	120	130/97
	89	126	8.9	2.2	6.0	120	139/113
	57	129	22.7	12.0	6.1	120	137/116
	106	128	4.9	1.0	4.1	120	146/116
	69	131	24.3	15.6	6.0	120	130/110
	107	128	5.2	0.9	4.6	120	154/120
4	119	139	17.6	8.4	13.3	150	128/114
	157	136	8.9	2.1	10.7	180	145/128
	112	138	16.2	5.6	11.9	180	134/118
	157	138	8.1	2.0	9.6	150	134/118
	133	140	15.4	4.2	14.9	180	134/118
5	103	158	16.7	6.4	10.6	165	148/121
	127	158	11.9	4.3	9.6	150	150/122
	112	160	17.4	7.1	11.5	150	152/124
	130	161	13.1	4.6	10.1	150	158/127
	127	159	17.2	7.8	11.9	150	150/120
	149	161	8.1	2.4	8.5	150	157/124
6	101	154	19.4	9.1	10.4	195	140/104
	106	158	16.6	8.2	8.9	195	140/104
7	144	160	20.1	16.7	4.9	145	113/79
	154	160	12.7	9.6	4.8	135	114/81
	153	160	20.0	16.8	4.9	130	112/80
8	126	191	21.7	14.8	8.7	170	121/99
	128	194	19.2	12.3	8.9	165	125/100
	138	196	18.0	11.4	9.1	150	129/101
	129	200	22.0	16.8	6.7	145	125/98
	139	200	13.4	8.2	7.2	140	129/100
9	117	152	20.5	13.6	8.1	135	129/91
	117	150	16.5	9.6	8.1	130	141/101
	104	153	20.4	13.0	7.7	140	147/102
	104	150	17.2	10.4	7.1	140	135/101
	107	150	20.5	13.1	7.9	140	125/93
10	133	176	14.0	5.9	10.8	190	139/110
	134	179	18.6	10.1	11.2	180	140/109
	138	178	14.0	6.2	10.8	180	145/114
	136	179	18.5	11.7	9.0	165	133/101
	139	179	13.2	6.0	10.0	165	141/106
11	122	180	24.3	14.7	11.7	180	120/95
	123	180	21.0	11.3	11.9	180	124/98
	118	180	18.4	8.8	11.3	180	114/90
12	131	111	17.0	13.4	4.7	Vent.	
	122	111	13.7	10.0	4.5	Fib.	
13	208	161	14.2	12.0	4.6	Vent.	
	196	159	12.5	10.3	4.3	Fib.	
	204	163	12.0	9.9	4.3		
	218	159	10.9	8.9	4.4		

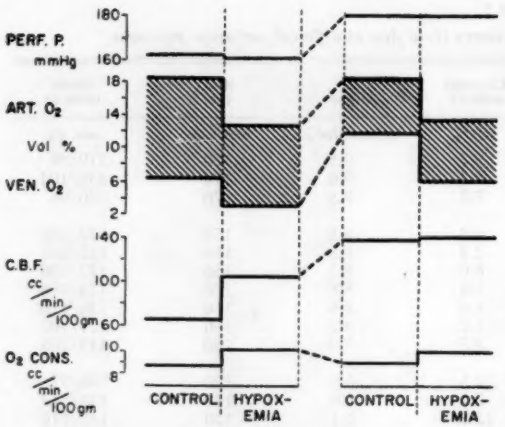

FIG. 2. EFFECT OF HYPOXEMIA ON CORONARY BLOOD FLOW AT TWO DIFFERENT PERFUSION PRESSURES

were roughly proportional to the reduction in coronary sinus oxygen content observed.

In Experiments 6 through 13, high coronary sinus oxygen levels were maintained during control periods by employing a high perfusion pressure. With moderate decreases in arterial oxygen content coronary blood flow remained relatively constant and coronary sinus oxygen content did not fall below 5.9 volumes per cent. Myocardial oxygen consumption, aortic pressure, and heart rate showed little change.

The importance of the oxygen supply to the myocardium, as reflected by the level of coronary sinus oxygen content, is illustrated in Figure 2, in which the effect of alterations in arterial oxygen content on coronary blood flow is seen in the same animal at different perfusion pressures. At a pressure of 160 mm. Hg and arterial oxygen content of 18.5 volumes per cent, coronary sinus oxygen content was 6.3 volumes per cent and coronary blood flow was 70 cc. per minute per 100 grams of heart muscle. When arterial blood containing 12.6 volumes per cent of oxygen was infused into the left coronary artery at the same pressure, coronary sinus oxygen content was reduced to 2.8 volumes per cent and flow rose to 105 cc. per minute. In the second half of the experiment, perfusion pressure was increased to 180 mm. Hg and at a control arterial oxygen level of 18.5 volumes per cent coronary flow was 136 cc. per minute and coronary sinus oxygen content 13.2 volumes per cent. When the arterial oxygen content was reduced to 11.7 volumes per cent at this higher perfusion pressure, coronary sinus oxygen content decreased to 6.0 volumes per cent and no significant change in coronary blood flow occurred.

The relationship between coronary blood flow and the oxygen content of venous blood is depicted for all experiments in Figure 3. The points represent the cardiac venous blood oxygen content during coronary perfusion with partially desaturated arterial blood plotted against the observed change in coronary blood flow. No change in coronary blood flow occurred at coronary sinus oxygen levels greater than 5.5 volumes per cent. However, below this point a direct relationship appears to exist between the reduction in coronary sinus oxygen content and the increase in coronary blood flow.

The absence of a direct relationship between arterial oxygen content and coronary blood flow is illustrated by Figure 4, in which arterial oxygen content of partially desaturated blood perfusing the coronary artery is plotted against the change in coronary blood flow observed during the infusion period. In Figure 4 the solid circles represent arterial oxygen values in experimental periods when coronary sinus oxygen was greater than 5.5 volumes per cent and the open circles when coronary sinus oxygen was below 5.5 volumes per cent. In the range of arterial oxygen content (10 to 15 volumes per cent), in which there is overlap of venous oxygen levels above and below 5.5 volumes per cent, all the points representing the higher coronary sinus oxygen levels are clustered about zero flow change, whereas points representing the lower sinus oxygen levels (at the same arterial oxygen level) are associated with increases in coronary blood flow.

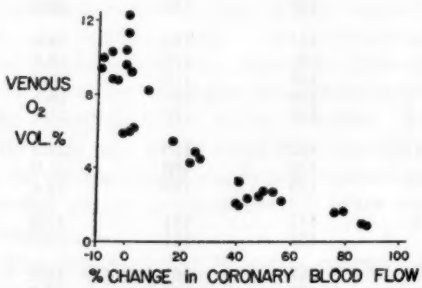

FIG. 3. RELATIONSHIP OF CORONARY SINUS BLOOD OXYGEN CONTENT TO CORONARY BLOOD FLOW

See text for discussion.

DISCUSSION

A reduction in the oxygen content of blood perfusing the coronary arteries and arterioles has no direct effect upon resistance within the vasculature of the heart, provided that the myocardium receives an adequate supply of oxygen. The fact that coronary blood flow increased when coronary sinus oxygen content fell below 5.5 [4] volumes per cent, regardless of the arterial oxygen content, suggests a causal relationship between venous oxygen content and coronary resistance in hypoxemia. A similar correlation has been found by Katz, Katz, and Williams (7). Since it is unlikely that dilation of venules or veins can produce reductions in coronary resistance of the order of magnitude observed in the present studies, and since venous oxygen content closely reflects tissue oxygen content, the coronary dilation of hypoxemia can probably be ascribed to reductions in oxygen content within the myocardium. This relative myocardial hypoxia could result in the local release of vasoactive metabolites, an idea that has been considered for over half a century. If arterial oxygen content is the primary factor responsible for resistance changes in the coronary circulation, then reduction in arterial oxygen content should have been equally effective in decreasing coronary resistance at different levels of oxygen within the myocardium.

Nervous and humoral mechanisms originating outside the heart have been eliminated in the present studies, since in the whole animal experiments only blood entering the left coronary artery was made hypoxic and in the fibrillating heart experiments a completely isolated heart was used. Alella (8) and Hackel and Clowes (9), who have observed marked increases in coronary blood flow in generalized hypoxia, found that cardiac denervation and/or adrenalectomy did not influence the response of the coronary vessels to hypoxia.

In 1925, Hilton and Eichholtz (2) challenged the hypothesis of the release of vasodilator substances during hypoxia and periods of increased myocardial metabolism and attributed the increased coronary flow of hypoxemia to a direct effect of the lowered arterial oxygen tension on the vessel walls. Their conclusions were based on the findings that 1) perfusion of the coronary arteries with fresh metabolite-free deoxygenated arterial blood or the intra-coronary administration of cyanide produced immediate increases in coronary blood flow, 2) myocardial oxygen consumption did not decrease with hypoxemia, 3) high concentrations of carbon dioxide and lactic acid produced only small increases in coronary flow, and 4) the progressive coronary vasodilation observed in the heart-lung preparation was not corrected by replacing the blood in the system with fresh blood. They also cited, as evidence against the "metabolite theory," experiments of Nakagawa (10) and Evans (11) in which increases in heart rate and cardiac output in the heart-lung preparation were attended by increases in oxygen consumption without concomitant increments in coronary blood flow.

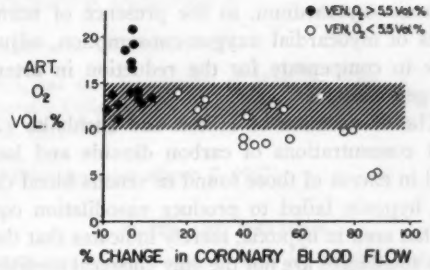

FIG. 4. RELATIONSHIP OF ARTERIAL BLOOD OXYGEN CONTENT TO CORONARY BLOOD FLOW
See text for discussion.

The rapidity of the response to fresh deoxygenated blood or to cyanide does not eliminate the possibility that vasoactive metabolites are released. Due to the large oxygen consumption and the high coefficient of utilization of oxygen in the normal beating heart, a sudden decrease in oxygen supply would produce an equally rapid reduction in myocardial oxygen tension. Since diffusion rates for substances of small or moderate molecular weights are high (12), it is quite conceivable that vasoactive materials released as a result of myocardial hypoxia could quickly reach the arterioles in effective concentrations.

The maintenance of myocardial oxygen consumption in hypoxemia could be attributed with equal justification to vasodilation induced by metabolites or to vasodilation induced by direct action of reduced arterial oxygen content on the vessel walls. There is no valid reason to exclude the possibility that vasoactive material released by

[4] Average value for coronary sinus oxygen in the normal dog (6).

hypoxic myocardium, in the presence of normal rates of myocardial oxygen consumption, adjusts flow to compensate for the reduction in arterial oxygen content.

The observation of Hilton and Eichholtz (2), that concentrations of carbon dioxide and lactic acid in excess of those found in venous blood during hypoxia failed to produce vasodilation equal to that seen in hypoxia, merely indicates that these two substances are not the only chemical mediators acting on the coronary vessels. It does not exclude other metabolites. Nor does the failure of fresh blood to reverse the progressive coronary dilation in the heart-lung preparation eliminate metabolites as playing a role in the vasodilation of hypoxemia.

Concerning the experiments of Nakagawa (10) and of Evans (11), it has now been firmly established in intact dogs, using precise methods for measuring flow, that coronary blood flow bears a direct relationship to myocardial oxygen consumption (13–15). In fact, this is the one function that can be consistently related to coronary blood flow, regardless of the means by which the rate of myocardial metabolism is increased.

The close correlation between myocardial oxygen consumption and coronary blood flow, the direct relationship between the duration of cardiac ischemia and the degree of reactive hyperemia (16), and the observations that reductions in arterial oxygen content in the absence of myocardial hypoxia do not increase coronary blood flow suggest that myocardial oxygen content and not arterial oxygen content is the critical factor in the regulation of coronary blood flow. Whether a decrease in myocardial oxygen content results in release of vasodilator substances remains to be proven.

SUMMARY

In experiments on open-chest dogs and on fibrillating heart preparations, reduction of oxygen content of arterial blood produced increases in coronary blood flow only when coronary sinus oxygen levels fell below about 5.5 volumes per cent. High perfusion pressures were employed in order to increase coronary flow to the extent that coronary sinus blood became relatively rich in oxygen. Under these conditions it was possible to demonstrate that a moderate lowering of arterial oxygen content does not decrease coronary resistance by a direct action on the vessel walls. Coronary vasodilation in hypoxemia appears to be related to myocardial hypoxia.

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RENAL FUNCTION IN WILSON'S DISEASE[1]

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Wilson's disease (hepatolenticular degeneration) is an hereditary disorder characterized by a variety of metabolic defects. There is excessive absorption of copper from the intestinal tract (1, 2) and, as a consequence, an increase in the total copper content of the body, particularly in the brain, liver (3) and kidneys (4). The serum ceruloplasmin, the main copper-carrying protein present in the serum, is abnormally low (5, 6). The total serum copper concentration also is lower than normal (6, 7), although the small fraction of copper bound to serum albumin, designated by Cartwright and his co-workers as the "direct-reacting copper," is increased (7). There is excessive urinary excretion of copper (8).

The finding of marked aminoaciduria in patients with Wilson's disease by Uzman and Denny-Brown in 1948 (9) revealed still another metabolic abnormality which occurs in many but not all cases (10). There may also be increased urinary urate excretion, accompanied by a moderate decrease in the serum urate level (11). Glycosuria, in the absence of a raised blood glucose concentration, is an occasional additional finding (12).

The occurrence of marked aminoaciduria, uricosuria and glycosuria in some patients with Wilson's disease suggests the possibility of renal involvement in this disorder (12). Little information is available concerning the nature and extent of the presumed renal defect (13, 14).

The present investigation was designed to afford a more composite analysis of discrete renal functions in Wilson's disease by applying simultaneous clearance techniques. A preliminary report of some of the results has appeared elsewhere (15).

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CASES AND METHODS

The nine patients studied were in varying stages of the disease (Table I). The manifestations of the disorder in these patients were predominantly neurological. None gave evidence of advanced cirrhosis of the liver and none had ascites. All of the patients had a decreased serum ceruloplasmin. In eight of the nine patients the total serum copper excretion was reduced. The urinary copper excretion was excessive in every instance. The daily urinary output of α-amino nitrogen was increased, or an increase could readily be induced by a high protein intake. All of the patients had received considerable quantities of BAL medication but this was discontinued for at least three days prior to the clearance study.

Eleven renal clearance studies were performed in these nine patients, using standard techniques (16). The glomerular filtration rate was measured by inulin clearance; renal plasma flow by clearance of para-aminohippurate; tubular excretory mechanisms in six instances by estimation of Tm_{PAH}; tubular reabsorptive mechanisms by clearances of amino acids, urate and phosphate, and in four patients also by estimation of Tm glucose. As a further measure of tubular excretory and reabsorptive capacities, the inhibiting effect of probenecid on tubular transport of PAH and urate was studied in seven patients. The excretion of bicarbonate and titratable acid was determined in four patients.

All experiments were performed in the morning with the patient in the fasting, post-absorptive state. To ensure adequate urine flow for the duration of the clearance experiments, liberal quantities of water were administered orally. Three or four urine collections were made by indwelling catheter in every experiment, each collection comprising a period of 20 minutes; in the experiments with probenecid, six 20-minute collections were made. When the determination of bicarbonate was to be included in the analysis, the customary air insufflation and bladder wash were omitted, and paraffin oil was used to prevent loss of CO_2. Urine pH determinations were made by means of a Cambridge pH meter as soon as each urine sample was procured.

Two patients (Cases Y.R. and L.A.) were hospitalized and maintained on a constant diet of 2,500 calories. After a control period of six days, ammonium chloride in syrup of cherry was administered for four successive days in doses of 10, 14, 14 and 16 Gm. per day, respectively. Observations were continued for five days after cessation of medication. The urine, preserved with tolu-

TABLE I

General description of the nine cases of Wilson's Disease studied

Name Sex Age	Approximate duration of overt disease	Clinical severity*	Osteomalacia or bone fragmentation	Albuminuria†
	years			*mg./24 hours*
M. F. Male 40	3	+++	0	<10
R. V. Male 13	4	+	0	<10
M. G. Male 33	5	+	+	180
C. P. Male 27	6	+	0	270
P. C. Female 36	7	++	+	<10
F. L. Male 35	8	+	+	20
L. A. Male 53	20	+	0	140
Y. R.‡ Female 37	23	++	+	
B. R.‡ Female 37	23	++	0	60

* Clinical severity: + Mildly incapacitated but employable, ++ Severely incapacitated, +++ Bedridden.
† Normal range (10 to 20 mg. per 24 hours).
‡ Uniovular twins.

ene, was collected daily throughout the 15-day period of study and the pH, titratable acid, and bicarbonate were determined.

Analytical methods. Inulin, para-aminohippurate, urate, phosphate, ammonia, titratable acid, and creatinine were determined by the standard procedures indicated elsewhere (17). In estimating glucose Tm, samples for inulin analysis were digested with baker's yeast before color development with diphenylamine, instead of the Roe method employed otherwise for determination of inulin. Glucose was determined by the Folin and Wu method. Plasma α-amino acid nitrogen was estimated by the method of Hamilton and Van Slyke (18), urinary α-amino acid nitrogen by the method of Van Slyke, MacFadyen, and Hamilton (19). Plasma and urinary bicarbonate were determined by Van Slyke's manometric technique. The urinary excretion of albumin was estimated by an immunological technique (20).

RESULTS

Except in one instance (Case R.V.), the *glomerular filtration rate,* as measured by the inulin clearance, was reduced (Table II). The reduction was moderate in Cases M.F., P.C., C.P., M.G., and F.L. (range, 76.2 to 91.9 ml. per min.), more pronounced in Cases L.A., Y.R., and B.R. (range, 48.9 to 69.0 ml. per min.).

As another indication of glomerular involvement, the urinary excretion of albumin was increased in Cases C.P., M.G., L.A., and B.R. (270, 180, 140, and 60 mg. per 24 hours, respectively). In the remaining four cases, the urinary excretion of albumin was within the normal range (< 10 to 20 mg. per 24 hours) by the immunologic method employed. The blood urea nitrogen was not elevated in any of the patients.

Renal plasma flow, as measured by clearance of para-aminohippurate, was consistently reduced. In six cases, C_{PAH} ranged from 453 to 342 ml. per min. In two patients (Cases L.A. and Y.R.), C_{PAH} was less than 250 ml. per min. (Table II). The filtration fraction, C_{inulin}/C_{PAH}, was somewhat higher than normal in most instances, with a mean of 0.238. The blood pressure was normal in every instance.

Tubular excretory capacity, as measured by Tm_{PAH}, was invariably reduced in the six patients examined (Table II). In four instances (Cases M.F., M.G., L.A., and Y.R.), the values obtained were below 40 mg. per min.; in Cases P.C. and F.L., Tm_{PAH} was 59.1 and 43.5 mg. per min., respectively.

Tubular reabsorptive mechanisms, in respect to α-amino nitrogen, urate, inorganic phosphate and glucose, were found to be impaired (Table II).

The $C_{\alpha\text{-}NH_2N}/C_{inulin}$ ratio in six of the nine cases was considerably increased. The highest values were found in the three cases of longest duration of overt disease (L.A., Y.R., and B.R.), in whom $C_{\alpha\text{-}NH_2N}/C_{inulin}$ was 15.8 per cent, 10.0 per cent and 16.8 per cent, respectively. (Normal range, 1.0 per cent to 3.0 per cent). The plasma α-amino nitrogen in these patients did not differ significantly from normal.

The plasma *urate* was less than 3.0 mg. per cent in six patients (2.0 mg. per cent in Case B.R.), and in the remainder varied between 3.0 and 3.9 mg. per cent—all below the minimum normal level

by the analytical method employed. (Normal range, 4.0 to 6.0 mg. per cent). C_{urate} was uniformly increased, in four instances exceeding 20 ml. per min. This increase is even more striking when the reduced glomerular filtration rate is taken into account; the mean C_{urate}/C_{inulin} was 28.0 per cent, range from 15.4 to 46.7 per cent.

The plasma *inorganic phosphate* was in the lower range of normal in five of the six cases examined (2.4 to 3.3 mg. per cent) and distinctly reduced in one instance (Case L.A., 1.5 mg. per cent). Phosphate clearance exceeded the normal in each of these six cases, ranging from 18.7 to 37.1 ml. per min., with a mean of 25.8 ml. per min. Calculation of the ratio, C_{PO_4}/C_{inulin}, emphasized the increase in phosphate clearance, the mean value obtained being 34.1 per cent.

The fasting blood *glucose* in the four patients in whom it was measured (Cases M.G., M.F., F.L., and C.P.) was at low normal levels (78, 84, 87 and 87 mg. per cent, respectively). The glucose Tm was measured in these four patients. In two, the results obtained were somewhat below the lower limit of the normal range (Cases M.F. and F.L., 272 and 246 mg. per min., respectively). In one instance (Case M.G.), the glucose Tm was reduced to 184 mg. per min. In Case C.P. the value obtained was strikingly low, 95 mg. per min. None of these patients had spontaneous glycosuria or hypoglycemia. Occasional spontaneous glycosuria was present in three cases (L.A., B.R., Y.R.).

Effect of probenecid on tubular transport mechanisms (Table III)

Probenecid is a potent inhibitor of renal tubular excretory systems (21), markedly suppressing tubular transport of PAH (17, 22); this effect is less pronounced if the transport mechanisms are deficient, the diminished effect thus serving as a further indication of the degree of impairment of tubular excretory mechanisms. In two patients with clinical manifestations of Wilson's disease of relatively short duration (approximately five years), probenecid given intravenously in a dosage of 26 to 32 mg. per Kg. body weight reduced Tm_{PAH} significantly, from 59.1 to a minimum of 22.6 (Case P.C.); and from 39.2 to a minimum of 29.5 mg. per min. (Case M.G.). This response

TABLE II

*Renal clearance studies in nine patients with Wilson's Disease**

Name	C_{In}	C_{PAH}	Tm_{PAH}	P_{a-NH_2N}	Ca_{-NH_2N}	P_{Urate}	C_{Urate}	P_{PO_4}	C_{PO_4}	TmG	$\frac{C_{In}}{C_{PAH}}$	$\frac{Ca_{-NH_2N}}{C_{In}}$	$\frac{C_{Urate}}{C_{In}}$	$\frac{C_{PO_4}}{C_{In}}$	Urine pH
	(ml./min.)	(ml./min.)	(mg./min.)	(mg. %)	(ml./min.)	(mg. %)	(ml./min.)	(mg. %)	(ml./min.)	(mg./min.)	(%)	(%)	(%)	(%)	
M. F.	91.9	422		3.5	2.9	3.5	15.3	2.4	28.0		21.9	3.1	16.7	30.4	6.6–6.8
	90.5					3.9	14.0						15.5		6.8–7.2
R. V.	107.0	453	37.9	4.8	4.9	2.4	32.4	3.3	37.1	272	23.6	4.6	30.2	34.7	7.1–7.3
M. G.	89.7	360	39.2	4.6	6.3	2.4	25.6	2.5	22.4		24.9	7.0	28.4	25.0	6.8–7.3
	79.0	342								184	23.1				
C. P.	85.7	345		4.4	2.5	3.7	13.2			95	24.8	2.9	15.4		6.6–7.2
P. C.	76.2	371	59.1	4.3	4.4	2.6	17.5	2.5	18.7		20.6	5.8	23.0	24.5	
F. L.	83.2	360	43.5	3.9	1.1	2.8	24.0			246	23.1	1.3	28.9		6.9–7.0
L. A.	48.9	199	14.5	4.4	7.7	3.0	18.7	1.5	28.8		24.6	15.8	38.2	59.0	7.7–8.0
Y. R.	63.5	233	21.5	4.9	6.4	2.5	16.2	2.5	19.5		27.3	10.0	25.5	30.7	
B. R.	69.0			3.6	11.6	2.0	32.2					16.8	46.7		7.2–7.5
Normal values	♂ 124±25.8 ♀ 108±13.5	654±163 592±153	79.8±16.7	3.4–5.5	1.0–3.0	4.0–6.0	8.0±2.0	3.0–5.0	10.0–12.0	350±50	19.2±3.5	1.0–3.0	7.0±2.0	10.0	

* The patients are listed in order of increasing duration of overt disease.
All clearance values are corrected to standard body surface area (1.73 M.²). All values are means of three or four 20-minute clearance periods.
All normal values except for α-amino acid nitrogen and urate are quoted from References 29 and 33.

TABLE III

Effect of probenecid on C_{In}, C_{PAH} (or Tm_{PAH}) and C_{Urate} in seven patients with Wilson's Disease

Name	Dosage of probenecid	C_{In}		C_{PAH}			Tm_{PAH}			C_{Urate}		
					After probenecid			After probenecid			After probenecid	
		Control*	After probenecid†	Control	Mean	Minimum	Control	Mean	Minimum	Control	Mean	Peak
	(mg./Kg.)											
M.F.	40, I.V.	91.9	91.8	422	243	193				15.3	35.7	38.0
R.V.	40, I.V.	107.0	129.0	453	279	212				32.4	51.6	58.9
F.L.	40, I.V.	65.3	65.8	341	201	175				14.3	34.7	38.4
M.G.	32, P.O.	89.7	84.4	360			39.2	32.9	29.5	25.6	25.7	34.3
P.C.	27, P.O.	76.2	71.7	371			59.1	32.9	22.6	17.5	26.7	32.4
L.A.	28, P.O.	48.9	52.2	199			14.5	11.5	8.6	18.7	22.1	24.1
Y.R.	26, P.O.	63.5	63.0	233			21.5	22.4	20.0	16.2	21.8	23.4
Means		77.5	79.7	405 (3 cases)	241	193	33.6	24.9	20.2	20.0	31.2	35.6

* All control values represent the means of three or four 20-minute periods. All control and post-medication values are corrected to standard body surface area of 1.73 M.²

† All values after probenecid represent the means of six or seven 20-minute periods.

was somewhat less marked, however, than that of a normal subject in whom probenecid in oral dosage of 20 mg. per Kg. body weight reduced Tm_{PAH} from 83.4 to 26.4 mg. per min. (17). In two patients with symptoms of some 20 years' duration (Cases L.A. and Y.R.: Tm_{PAH} 14.5 and 21.5 mg. per min., respectively), probenecid elicited, at most, an equivocal further depression of Tm_{PAH}. In three patients (Cases M.F., R.V., and F.L.), whose clinical symptoms were of relatively short duration, and whose initial C_{PAH} was but moderately reduced (422, 453, 341 ml. per min.), there was marked further suppression of C_{PAH} by probenecid. The response did not seem to differ in degree from that of (gouty) subjects with intact kidney function (17).

Probenecid markedly suppresses tubular reabsorption of urate and thus increases urate clearance in the intact kidney. In 10 gouty subjects with little or no renal damage, probenecid in oral dosage of 20 to 30 mg. per Kg. body weight evoked an increase in C_{urate} of approximately 300 per cent, from a mean of 8.5 ml. per min. to peak values averaging 32.9 ml. per min. (range, 10.2 to 51.3 ml. per min.) (17). In the seven patients with Wilson's disease examined, there was an increase in C_{urate} of approximately 75 per cent after intravenous administration of probenecid in comparable dosage, from a mean of 20 ml. per min. to a mean peak of 35.6 ml. per min. This poor percentile response is attributable in part to the high initial C_{urate}. The results in the two patients with symptoms of longest duration (Cases L.A. and Y.R.), in whom C_{urate} increased from 18.7 to a peak of only 24.1 ml. per min. and from 16.2 to 23.4 ml. per min., respectively, suggest deficient tubular response to probenecid. The findings in these two patients were comparable to those obtained in the Fanconi syndrome, in which there was no response to probenecid (17, 23).

Effect of ammonium chloride acidosis

It was observed in the course of the foregoing renal clearance studies that the urine pH ranged from 6.6 to 8.0 (Tables II and IV). This prompted measurement of urinary bicarbonate excretion which, in the four patients studied (Cases M.F., M.G., C.P., and F.L.), was found to vary from 0.088 to 0.034 mM per min. at levels of plasma bicarbonate ranging from 25.0 to 20.7 mM

TABLE IV

Bicarbonate excretion in four patients with Wilson's Disease

Name	Urine flow	C_{IN}	Plasma $BHCO_3$	Urine pH	Urine $BHCO_3$	Urine H^+	$BHCO_3$ filtered	$BHCO_3$ reabsorbed	$BHCO_3$ filtered	$BHCO_3$ reabsorbed
	(ml./min.)	*(ml./min.)*	*(mM/L.)*		*(mM/min.)*	*(mM/min.)*	*(mM/min.)*	*(mM/min.)*	*(mM/100 ml. glomerular filtrate)*	
M. F.	9.8–16.1	90.5	25.0	6.8–7.1	0.088	0.002	2.26	2.17	2.50	2.40
F. L.	3.2–14.1	83.2	20.7	6.9–7.0	0.046	0.013	1.72	1.67	2.07	2.01
C. P.	5.8– 9.4	85.7	22.0	7.0	0.062	0.010	1.89	1.83	2.20	2.14
M. G.	4.0– 9.3	79.0	22.0	6.8	0.034	0.010	1.74	1.71	2.20	2.16

per L. The results (Table IV) suggest that tubular reabsorption of bicarbonate may not be quite as efficient in some patients with Wilson's disease as in the normal subject.

Ammonium chloride acidosis was produced in Patients Y.R. and L.A. In the six-day control period, Case Y.R., in whom overt Wilson's disease was of longest duration, excreted 18.8 to 26.4 mM bicarbonate per day (mean, 21.7 mM per day) at a mean plasma bicarbonate level of 23.1 mM per L. The urine pH varied from 6.9 to 7.2, the titratable acid averaged 4.4 mEq. per day. With induction of acidosis (plasma bicarbonate reduced to a minimum of 14.6 mM per L.), the urine pH fell to a low of 4.9, the titratable acid increased to a maximum of 38.0 mEq. per day, bicarbonate disappeared from the urine.

A similar response was noted in Case L.A. In the six-day control period, the excretion of bicarbonate varied from 11.1 to 16.5 mM per day (mean, 14.1 mM per day) at a mean plasma bicarbonate level of 27.6 mM per L. The range of urine pH was 6.2 to 7.0, the titratable acid averaged 11.7 mEq. per day. Administration of ammonium chloride reduced the plasma bicarbonate level to a minimum of 19.1 mM per L. and the urine pH to a low of 5.1. The titratable acid increased to a maximum of 41.9 mEq. per day, urinary bicarbonate excretion became negligible.

The urinary excretion of ammonia in Cases Y.R. and L.A. was within normal limits (means, 25.2 and 22.7 mEq. per day, respectively) in the control period.

DISCUSSION

The results of these renal clearance studies confirm and amplify previous reports indicating impairment of tubular transport systems and renal hemodynamics in patients with Wilson's disease, at least in its more advanced stages. Some hitherto unrecorded abnormalities are described.

Hodges and co-workers (13, 14) noted marked and consistent reduction in C_{PAH} and equivocal decline in C_{inulin} in their study of four cases of Wilson's disease. The present investigation corroborates the substantial reduction in renal plasma flow and discloses distinctly decreased glomerular filtration rate in most instances. The degree of impairment seemed roughly to parallel the severity or duration of overt disease in this series of patients, suggesting progressive deterioration of the renal vascular bed.

The tubular secretory capacity, as measured by Tm_{PAH}, was found to be reduced, strikingly so in the more advanced cases. Associated with this decline was a diminishing effect of probenecid in eliciting a further reduction in Tm_{PAH}.

Impairment of renal tubular reabsorptive activities in Wilson's disease has been suspected for some time. The excessive aminoaciduria, for example, has been attributed to inadequate tubular reabsorption (12, 24). This interpretation is supported by the high values obtained for $C_{\alpha\text{-}NH_2\text{-}N}$, and particularly for $C_{\alpha\text{-}NH_2\text{-}N}/C_{inulin}$, in the face of normal plama levels of α-amino nitrogen. Similarly, diminished tubular reabsorption of filtered urate, suggested by Bishop, Zimdahl, and Talbott (11) and Mahoney, Sandberg, Gubler, Cartwright, and Wintrobe (25), to explain the uricosuria and low plasma urate levels noted by them in patients with Wilson's disease, is indicated by the augmented urate clearance ratios consistently observed in the present study. The capacity of probenecid further to increase C_{urate}/C_{inulin} decreased as the tubular transport systems for urate deteriorated with progress of the disease, until the drug finally was quite ineffective, as in the Fanconi syndrome.

In all six of the patients so studied, an increase in C_{PO_4} was observed, and in five of these the serum inorganic phosphate was in the low normal range or reduced. Cooper, Eckhardt, Faloon, and Davidson (12) described a patient with Wilson's disease who was reputed to have had hypophosphatemia prior to their study. Warnock (26) reported a patient with Wilson's disease with spontaneous fractures, demineralization of bone, and a plasma inorganic phosphate level of 2.5 mg. per cent. The low serum phosphate and increased renal clearance of phosphate may be responsible, in part, for the occasional occurrence of osteomalacia in this disease (12, 26, 27).

The observation that glycosuria may be present in some patients with Wilson's disease has been somewhat amplified in the present study, in which the tubular reabsorptive capacity for glucose was measured. It is noteworthy that, although spontaneous glycosuria was not usually present, the maximum tubular capacity to reabsorb glucose was reduced in varying degree in all four cases studied. These findings emphasize the fact that failure to find increased urinary excretion of glucose in Wilson's disease does not preclude a very considerable defect in the capacity of the renal tubules to transport glucose across the tubular epithelium.

The tendency to excrete urine of high pH, due to renal excretion of small quantities of bicarbonate at plasma bicarbonate levels which should not be associated with urinary loss (28), suggests that there may be slight impairment of tubular reabsorption of bicarbonate in advanced stages of Wilson's disease. When ammonium chloride acidosis was induced, there was a prompt decline in urine pH, increase in titratable acidity, and disappearance of urinary bicarbonate.

The precise mechanisms involved in the impairment of tubular functions observed in Wilson's disease must remain speculative so long as the normal mechanisms of renal tubular transport are poorly understood (29). The hypothesis that the renal lesion is a primary effect of the abnormal gene on the functional integrity of the renal tubule receives little support from this study. That the abnormal gene may result in an anatomical defect of the renal tubule, not necessarily related to the described functional abnormalities, is suggested by the preliminary observations of Soothill and Kark (30). They have observed a deformity in the proximal convolution of the tubule in renal biopsy specimens obtained from patients with Wilson's disease. It was not apparent to them whether this abnormality was primary, as in the Fanconi syndrome, or whether this was due to a local change resulting from Wilson's disease. Darmady (31), however, was unable to discern any anatomical defect in the proximal renal tubules in the kidneys of patients who had died of Wilson's disease. But although the details of the pathogenesis of the disease and its varied manifestations remain largely unknown, a plausible hypothesis to explain the renal lesions can be tentatively suggested as a result of this study. The deficiency of serum ceruloplasmin in Wilson's disease is associated with an increase in the copper loosely bound to serum albumin. This loosely bound copper can be deposited in any tissue where there are substances which can compete successfully with albumin for the copper ion. With increased deposition of copper in the kidney, the metal may interfere with essential enzyme systems responsible for the transport of materials across the tubular epithelium, such as is presumed to occur in chronic poisoning with other divalent metals (32). Initially, the brunt of the damage falls predominantly on the proximal renal tubule, resulting in defective transport of PAH, phosphate, urate, amino acids, and glucose, in these respects resembling somewhat the defects occurring in Fanconi syndrome. This emphasizes the desirability of excluding Wilson's disease in patients with defective renal tubular functions.

With progression of the renal lesion, other functions of the kidney are disturbed and the functional abnormality is no longer restricted to any anatomical segment of the renal tubule. A decreased glomerular filtration rate, and a tendency to excrete an alkaline urine, indicate more widespread renal damage.

SUMMARY

1. Eleven renal clearance studies were carried out in nine patients with Wilson's disease in various stages of the disorder.

2. The inulin clearance was below the lower limits of normal in eight patients. C_{PAH} was decreased in every instance.

3. Tubular secretory capacity, as measured by Tm_{PAH}, was reduced in the six patients examined. When Tm_{PAH} was very low, probenecid elicited little further decrease.

4. Tubular reabsorption of α-amino nitrogen, urate, phosphate, and glucose was defective, as indicated by the generally increased clearance ratios. Serum inorganic phosphate levels were in the lower range of normal or reduced, and may account for the osteomalacia occasionally encountered in Wilson's disease. The blood glucose was in the low normal range. The Tm glucose tended to be low in the four patients so studied, probably explaining the occasional glycosuria in this disorder. Serum urate levels were consistently low. Probenecid elicited a uricosuric response in most instances but this was least pronounced in those subjects who initially had the most impaired tubular reabsorption of urate.

5. The urine pH tended to be more alkaline than normal, owing to slight urinary excretion of bicarbonate at plasma bicarbonate levels not ordinarily associated with renal loss of bicarbonate. The response of two subjects to ammonium chloride-induced acidosis was essentially normal in respect to mechanisms for acidification of the urine.

6. The results indicate progressive deterioration of certain discrete tubular functions, and also of glomerular filtration and renal plasma flow, with advance of the disease. It is suggested that these renal changes may reasonably be ascribed to the deleterious effects of the accumulation of copper in the kidneys; such effects are known to accompany the deposition of other heavy metals in this organ. In this view the renal abnormalities described are secondary to a disturbance in copper metabolism rather than a direct consequence of the abnormal gene on renal function.

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SEROTONIN AND HISTAMINE RELEASE DURING ANAPHYLAXIS IN THE RABBIT

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The release of physiologically active substances in animal tissues during anaphylaxis has been a subject of considerable interest for many years. Most of this interest has centered on the release of histamine from a bound to a free form, the latter being capable of producing marked physiological changes (1). However, despite the toxicity which can be attributed to histamine, attempts to explain all the alterations seen in anaphylaxis on the basis of histamine release alone have been unsatisfactory (2).

Many of the effects produced by serotonin (5-hydroxytryptamine) in animals (3) resemble the manifestations of hypersensitivity reactions. In addition, patients with malignant carcinoid, a syndrome (4) in which there is present an excessive amount of serotonin, frequently show symptoms resembling those of an allergic process. These findings have suggested that this amine may play a role in anaphylaxis and other allergic phenomena.

Serotonin is found in the blood platelets of animals and man (5). Anaphylaxis is known to produce profound changes in these blood cells (6). Recently, Humphrey and Jaques (7) showed that both serotonin and histamine were released from platelets upon addition of purified antigen and antibody to normal rabbit platelets suspended in plasma. Also, Herxheimer (8) reported that in guinea pigs an interrelationship exists between desensitization and tolerance to serotonin.

The present study was designed to determine whether serotonin is released during anaphylaxis. The experiments outlined below for serotonin are similar to those that were previously used to demonstrate the changes in blood histamine which occur during anaphylactic shock (9–12). Simultaneous determinations for both serotonin and histamine were carried out for comparative purposes. In this work, it has been shown that both amines appear in a free form in the plasma when a specific antigen is injected into sensitized rabbits.

MATERIALS AND METHODS

The rabbit was chosen for these experiments for several reasons: (a) the large amounts of both serotonin and histamine carried in the blood platelets, (b) the ease of obtaining serial blood samples, and (c) the ability of the rabbit to produce adequate amounts of antibody.

Male rabbits (1.5 Kg.) were sensitized to either egg albumin or horse serum. Those sensitized to egg albumin were given 10 ml. of a 10 per cent solution in saline intraperitoneally every other day for four injections. Those sensitized to horse serum were given 1 ml. of the undiluted serum intraperitoneally every day for six days. After three to five weeks, the rabbits were used as indicated. At this time, complement fixation antibody titers were high.

Siliconized glassware was employed wherever whole blood had to be handled. Blood for the *in vitro* studies was obtained by intracardiac puncture. Blood samples for the *in vivo* studies were obtained by means of a plastic tube inserted into the carotid artery.

Both serotonin and histamine were determined in all samples of plasma and whole blood. For plasma, 2 ml. were treated according to the method of McIntire, Roth, and Shaw (13) for the isolation of histamine. The method utilizes cotton acid succinate as a cation exchange medium after extraction of amines into n-butanol. It was found that histamine is removed from the butanol solution by the cotton acid succinate whereas serotonin is not. This procedure gives a convenient method for separating serotonin and histamine. Serotonin was then determined spectrophotofluorometrically (14), and histamine by modification of the methods of Lowry, Graham, Harris, Priebat, Marks, and Bregman (15) and McIntire, White, and Sproull (16). For assay of these amines in whole blood, 1 ml. was diluted with 7 ml. of water. After hemolysis, 1 ml. of 10 per cent zinc sulfate solution, and 0.5 ml. of 1 N sodium hydroxide were added to deproteinize the blood. After centrifugation, 1 ml. of the supernatant was taken for spectrophotofluorometric assay of serotonin; the remainder of the supernatant was assayed for histamine as described for plasma. The procedures employed in this study were convenient because both serotonin and histamine could be determined on the same deproteinized blood sample. The details of these methods will be published in a separate communication.

RESULTS

In vitro studies

Table I shows that upon addition of a specific antigen to the whole blood of sensitized rabbits, serotonin, as well as histamine, were liberated into the plasma. The values for serotonin and histamine in the experimental plasma samples were five to eight times greater than the values for the control plasmas, handled in the same manner.

Release into plasma in vivo

Results obtained with horse serum and egg albumin in intact rabbits are shown in Figures 1 and 2. In every case, serotonin and histamine were found in the plasma following the injection of the specific antigen. Both amines appeared almost immediately, the maximum values being reached within one or two minutes. The serotonin levels were consistently lower and persisted for a shorter period of time than the histamine levels.

In an attempt to estimate the possible quantitative significance of the increased amounts of these plasma amines appearing during anaphylaxis, solutions containing both serotonin and histamine were injected rapidly into the marginal ear vein of normal rabbits, and the plasma levels of serotonin and histamine determined. The results in Figure 3 show that under these conditions, too, serotonin and histamine disappear rapidly from plasma. In order to produce levels as high as those found dur-

TABLE I

Release of serotonin and histamine in rabbit blood in vitro *

Rabbits	Number	Serotonin (γ/ml.)		Histamine (γ/ml.)	
		1:100 Horse serum	Isotonic saline	1:100 Horse serum	Isotonic saline
Sensitized to horse serum	1	3.0	0.4	2.5	0.4
	2	2.6	0.2	3.9	1.0
	3	1.4	0.2	2.0	0.7
	4	5.0	0.9	3.2	0.9
	5	2.7	0.3	3.2	0.4
	6	2.6	0.1	3.5	0.4
	Average	2.9	0.4	3.1	0.6
Normal† controls	1	0.1	0.3	0.8	1.1
	2	0.1	0.1	0.6	0.6
	3	0.3	0.3	0.8	0.3
	4	0.8	0.5	0.8	0.4
	Average	0.3	0.3	0.8	0.6
		1:100,000‡ Egg albumin	Isotonic saline	1:100,000‡ Egg albumin	Isotonic saline
Sensitized to egg albumin	1	5.6	0.7	3.0	0.9
	2	4.5	0.3	1.9	0.5
	3	5.7	0.7	3.1	0.4
	4	5.1	0.8	4.0	0.5
	Average	5.2	0.6	3.0	0.6
Normal† controls	1	0.4	0.3	0.6	0.5
	2	0.4	0.8	0.4	0.3
	3	0.5	0.7	0.5	0.8
	Average	0.4	0.6	0.5	0.5

* Whole blood was incubated at 37°C. for 30 minutes with 50 per cent by volume of saline solution (1:100 horse serum or 1:100,000 egg albumin) of the antigen. The tubes were swirled every five minutes. After centrifugation, the diluted plasma was analyzed for serotonin and histamine. The values were calculated on the basis of the original plasma.

† Some of the control levels for serotonin and histamine in these experiments are higher than usually found. This is presumed to be due to the excessive handling of the blood in setting up and carrying out the incubations with subsequent breakage of some of the platelets to release these amines into the plasma.

‡ This dilution of egg albumin was used because more concentrated solutions caused release of serotonin and histamine in normal blood.

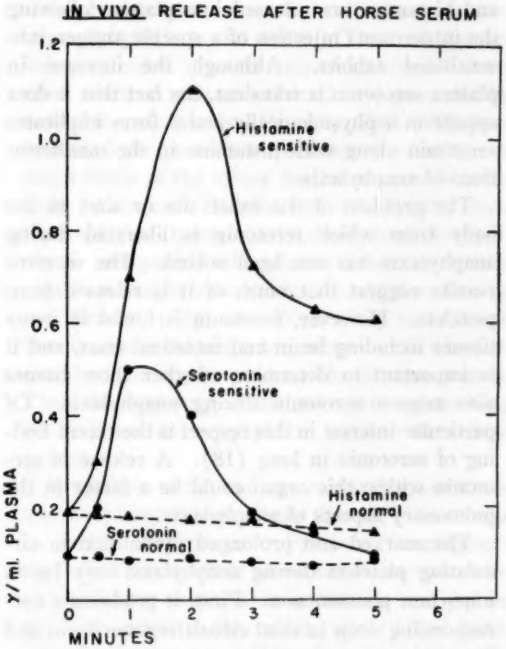

FIG. 1. *IN VIVO* RELEASE AFTER HORSE SERUM

Undiluted horse serum (1 ml. per Kg.) was injected intravenously into both sensitized and normal control rabbits, anesthetized with Nembutal® and ether. Plasma serotonin and histamine values were determined at the times indicated. The values are the averages of four sensitized animals and six normal controls.

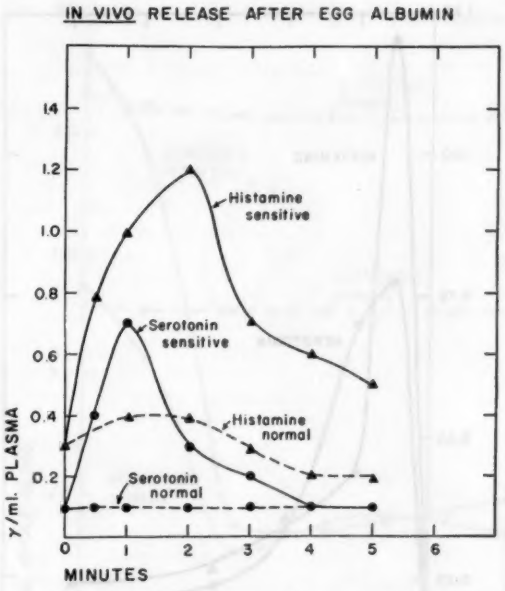

FIG. 2. *IN VIVO* RELEASE AFTER EGG ALBUMIN

Ten per cent egg albumin solution in saline (2 ml. per Kg.) was injected into both sensitized and normal control rabbits anesthetized with Nembutal® and ether. Plasma serotonin and histamine values were determined at the times indicated. The values are the averages of three sensitized animals and three normal controls.

ing anaphylaxis, it was necessary to inject at least 50 γ per Kg. of histamine and 150 γ per Kg. of serotonin. When 50 γ per Kg. of serotonin were injected, no increase in plasma serotonin could be detected.

The rabbit, during anaphylaxis, may not show many objective signs of toxicity despite the fact that chemical changes do occur. However, the most prominent toxic manifestation produced both by anaphylaxis and by injection of serotonin and histamine was respiratory in nature as shown by rapid, labored breathing. The effects following anaphylaxis were prolonged, whereas those following injection of the amines were transient.

Effect of anaphylaxis on serotonin and histamine in whole blood

Although the plasma levels of both amines were found to be increased as a result of anaphylactic shock, the total amounts of circulating serotonin and histamine were markedly diminished. This drop in total circulating amines resulted from a disappearance of blood platelets from the circulation. The experiments, shown in Figure 4 and Table II, indicate that following the injection of the antigen [1] into sensitized animals, the whole blood histamine and serotonin levels fell rapidly, as did the number of circulating platelets and leucocytes. Minimum values were reached in four minutes. The reduction in platelets persisted for one to two hours. At the end of this time the platelet counts had returned to values equal to those of the control blood samples, but the diminution of the amines persisted for at least four hours,

[1] For these experiments horse serum diluted 1:100 or 1:1,000 with saline was used since the animals often died too soon following injection of the undiluted horse serum. The diluted antigen, although very active in lowering the platelet count, increased plasma amine levels only slightly.

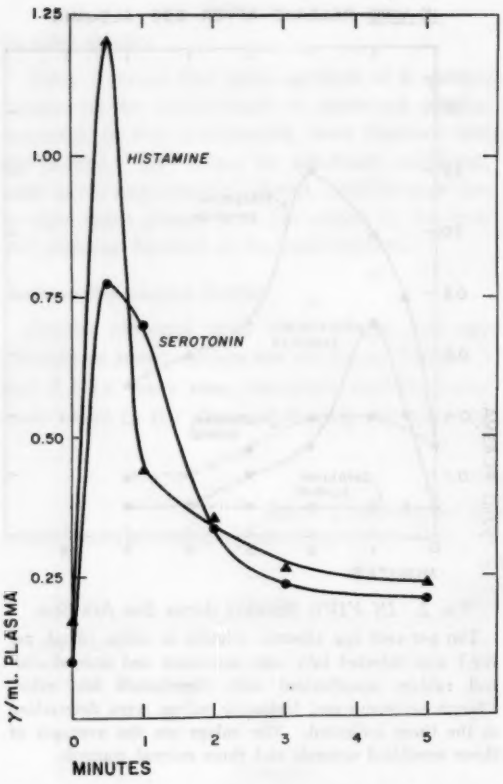

FIG. 3. PLASMA SEROTONIN AND HISTAMINE FOLLOWING INJECTION OF THE AMINES

Histamine 50 γ per Kg. and serotonin 150 γ per Kg. were injected into a marginal ear vein of a normal rabbit. Plasma was taken, after centrifugation, from blood obtained by means of a plastic catheter inserted into a carotid artery.

suggesting that there had been a release of serotonin and histamine from the platelets.

DISCUSSION

The finding that serotonin is released from platelets when antigen is added to blood from sensitized animals is in agreement with the results of Humphrey and Jaques (7), although the two experiments were not carried out in the same manner. The demonstration that histamine is released during antigen-antibody interaction is in agreement with the findings of many previous investigators (9, 10, 17).

The *in vivo* results show that both serotonin and histamine are released into plasma following the intravenous injection of a specific antigen into sensitized rabbits. Although the increase in plasma serotonin is transient, the fact that it does appear in a physiologically active form implicates serotonin along with histamine in the manifestations of anaphylaxis.

The problem of the exact site or sites in the body from which serotonin is liberated during anaphylaxis has not been solved. The *in vitro* results suggest that much of it is released from platelets. However, serotonin is found in many tissues including brain and intestinal tract, and it is important to determine whether these tissues also release serotonin during anaphylaxis. Of particular interest in this respect is the recent finding of serotonin in lung (18). A release of serotonin within this organ could be a factor in the pulmonary aspects of anaphylaxis.

The marked and prolonged diminution in circulating platelets during anaphylaxis may be an important phenomenon. First, it produces a corresponding drop in total circulating serotonin and histamine in the rabbit, although as has been pointed out above, the amounts of these free amines in the plasma are increased. Secondly, platelets are thought to play an important role in inactivating free serotonin and other amines (19) found in blood. Consequently, the paucity of circulating platelets, at a time when large amounts of amines are being released into the blood, may be an important factor in anaphylaxis.

TABLE II

Whole blood serotonin, histamine, platelets, and leucocytes during anaphylaxis *

Time	Serotonin (γ/ml. of whole blood)	Histamine (γ/ml. of whole blood)	Platelets (No./cu. mm.)	Leucocytes (No./cu. mm.)
Control	4.5	2.3	350,000	5,000
4 min.	0.8	0.6	<10,000	700
30 min.	1.2	0.9	75,000	3,200
1 hour	2.1	0.9	185,000	7,800
2 hours	3.0	1.2	385,000	10,200
3 hours	3.0	1.1	330,000	13,500
4 hours	2.9	1.1	265,000	15,000

* Whole blood serotonin and histamine levels, and platelet and leucocyte counts were determined at specific times following the injection of diluted horse serum (1:100 in saline; 1 ml. per Kg.) into sensitized rabbits. The values given represent the average of two experiments.

It is evident that serotonin release occurs during anaphylaxis in the rabbit. These studies are being extended to other animal species to determine whether serotonin liberation is generally involved in anaphylaxis. Recently, Fink (20) demonstrated the possible importance of serotonin in anaphylaxis in the mouse by the *in vitro* Schultz-Dale technique. Pallotta and Ward (21) have reported that lysergic acid diethylamide, a serotonin antagonist, will protect sensitized guinea pigs from anaphylactic shock. In addition, Rowley and Benditt (22), and West (23) have found that serotonin release is important in the anaphylactoid edema of rats produced by foreign protein. These findings suggest that serotonin release may be a factor in anaphylaxis in several animal species.

In addition to the findings concerning serotonin, this study may help to clarify the role of histamine in rabbit anaphylaxis. Previous investigators have shown that whole blood histamine values increase during anaphylaxis in the dog and guinea pig (24), while in the rabbit, whole blood histamine levels decrease (11). Because of this difference, it has even been suggested that histamine release might not be an important factor in anaphylaxis in the rabbit. It was only recently that Schachter (12) found that histamine was released into plasma during anaphylaxis in the rabbit. The present findings confirm not only the drop in the whole blood histamine, but also its release into plasma. An unusual feature about rabbits is that their platelets contain a much greater amount of histamine than do those of the dog or guinea pig. Consequently, a marked decrease in the number of platelets in rabbits causes a correspondingly marked decrease in the whole blood histamine level. This drop in whole blood histamine is so great that it obscures the transient rise in plasma histamine. In the other laboratory animals the amount of histamine present in their platelets is smaller than the amount released into plasma during anaphylaxis.

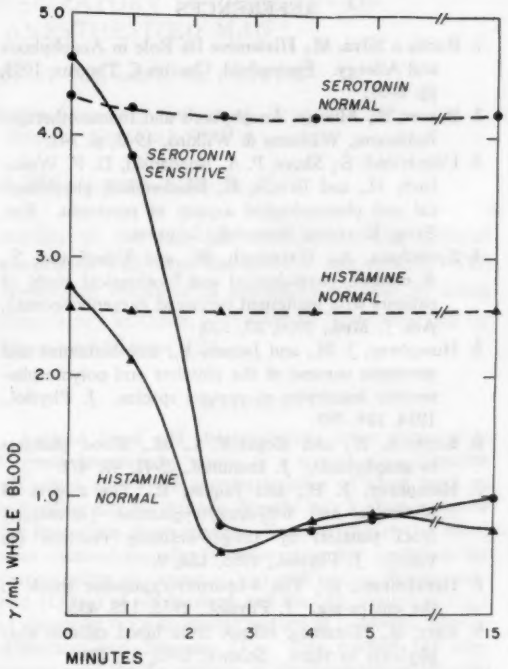

FIG. 4. WHOLE BLOOD SEROTONIN AND HISTAMINE DURING ANAPHYLAXIS

Diluted horse serum (1:100 or 1:1,000 in saline; 1 ml. per Kg.) was injected into sensitized and normal control anesthetized rabbits. Whole blood serotonin and histamine levels were determined at the times indicated. The points represent the average values obtained on seven sensitized and five control animals.

SUMMARY

1. Both serotonin and histamine were released into the plasma when a specific antigen was added to the whole blood from a sensitized rabbit.

2. Following injection of antigen into sensitized rabbits free serotonin and histamine appeared in the plasma. Maximum levels of serotonin and histamine were reached in one minute and declined to control values within two to three minutes. Histamine rose to higher levels than serotonin and declined more slowly.

3. Anaphylaxis was also accompanied by a marked fall in the levels of serotonin and histamine in whole blood due to a decrease in the number of circulating platelets. The diminished values were maintained for over two hours.

ACKNOWLEDGMENT

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THE IMMEDIATE EFFECTS OF RESPIRATORY DEPRESSION ON ACID–BASE BALANCE IN ANESTHETIZED MAN [1]

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The earliest report of the immediate effects of elevated carbon dioxide tension on acid-base balance during anesthesia was published by Henderson and Haggard in 1918 (1). They observed that an increase in CO_2 tension of the pulmonary air resulted in an increase in the CO_2 capacity of the blood of narcotized dogs. They postulated that this change was due to the passage of alkali from tissues into blood and resulted in the maintenance of the hydrogen ion concentration of the blood at a normal or nearly normal level. In 1932, Shaw and Messer (2) reported similar experiments with contrary results. They observed that dogs breathing high concentrations of carbon dioxide invariably developed a significant reduction of CO_2 capacity of the blood. When these experiments were repeated in cats, the alteration was even more striking. They suggested that the elevated blood bicarbonate, resulting from CO_2 retention, migrated into tissues as a result of physical forces which were regulated by the ionic concentration gradient.

Recently, Giebisch, Berger, and Pitts (3) and Elkinton, Singer, Barker, and Clark (4) have observed responses to CO_2 breathing in anesthetized dogs and unanesthetized man, respectively, which confirm the observations of Henderson and Haggard. On the other hand, Himwich, Gildea, Rakieten, and DuBois (5) consistently observed a reduction of CO_2 capacity in unanesthetized humans breathing 5 per cent CO_2 for 30 minutes. The same authors obtained this response in four of six experiments on dogs during CO_2 inhalation of 30 minutes or less, but obtained the opposite response in animals breathing CO_2 for periods longer than 55 minutes. Observations made on man during studies of the acidosis associated with general anesthesia will form the basis of this report. The immediate response of anesthetized man to acute respiratory acidosis is similar to that observed by Shaw and Messer (2).

METHODS

Arterial blood pH determinations were obtained at frequent intervals by means of a special glass electrode pH meter (6) which permits transfer of blood from an indwelling arterial cannula directly into a glass electrode for immediate measurement. Samples of arterial blood were obtained periodically under anaerobic conditions for determination of oxygen (O_{2_b}) and carbon dioxide content (CO_{2_b}) of whole blood, and the CO_2 content (CO_{2_p}) of plasma by the manometric method of Goldstein, Gibbon, Allbritten, and Stayman (7). This method has been proven valid for the analysis of blood samples which contain the commonly used volatile anesthetic agents (8). The hematocrit (Ht) was determined by centrifugation in Sanford-Magath tubes or by a calculation based on the difference between the content of carbon dioxide in the whole blood and the plasma, using the nomogram of Van Slyke and Sendroy (9). Oxygen saturation (Sat.) was calculated from the oxygen content of whole blood and an oxygen capacity based on the hematocrit according to the relationship given by the nomogram of Van Slyke and Sendroy. CO_2 tension (P_{CO_2}) was calculated from arterial plasma pH and CO_2 content using the Henderson-Hasselbach equation and the constants: $pK' = 6.10$, $\alpha_{CO_2} = 0.0301$. The amount of cation in excess of the non-buffer anion of whole blood, the buffer base (BB), was read from the nomogram of Singer and Hastings (10).

In most instances, the analytical procedures were completed within two hours of the drawing of the blood samples. All analyses were performed in duplicate. The reproducibility (one standard deviation) of the analytical methods as determined in collateral studies (6, 8) were as follows: pH ± 0.01; CO_{2_b} ± 0.12 mM per L.; CO_{2_p} ± 0.09 mM per L.; O_{2_b} ± 0.18 vol. per cent Ht ± 4 per cent.

A test of the reliability of the methods was conducted on arterial blood of five healthy, unanesthetized, adult volunteers (Table I). For comparison, P_{CO_2} was determined directly by a semi-micro method (11). The mean P_{CO_2} calculated from pH and CO_{2_p} was 2.2 mm.

[1] This work was supported by a grant from the National Tuberculosis Association, made possible by a special bequest from the estate of Grace Velie Harris. Preliminary report: Bull. New York Acad. Med., 1952, 28, 543.

TABLE I

Direct and indirect measurements of P_{CO_2} of arterial blood of healthy, unanesthetized adult subjects *

Subj.	1 CO_{2c} *mM/L.*	2 pH	3 P_{CO_2} direct *mm. Hg*	4 P_{CO_2} indirect *mm. Hg*	5 pH indirect	6 BB *mEq./L.*	7 BB *mEq./L.*
D. H.	26.65	7.39	41.6	41.7	7.39	48.1	48.1
C. W.	26.70	7.37	(29.3)	43.1		47.8	
F. H.	26.94	7.37	42.5	44.1	7.39	48.8	49.4
A. B.	26.04	7.36	39.8	43.6	7.40	47.0	48.2
A. B.	26.04	7.37	40.6	42.7	7.39	47.7	48.0
M. B.	26.58	7.37	39.8	43.5	7.41	46.8	48.0
Means	26.49	7.371	40.9	43.1	7.396	47.7	48.3

* The first three columns present measured values; the last four columns contain derived values. The values in columns 4 and 6 were derived from data in columns 1 and 2; the values in columns 5 and 7 were derived from data in columns 1 and 3. The buffer used as a standard for pH measurements was represented by the manufacturer to have a pH of 6.96 ± 0.02 at 37° C. Two independent studies were performed on subject A. B. at different times. The value for direct P_{CO_2} in the second row was assumed to reflect an error in method and is not included in the average.

Hg higher than the mean direct P_{CO_2}. The mean BB, based on pH and CO_{2_p}, was 47.7 mEq. per L., as compared with a mean of 48.3 mEq. per L. based on direct P_{CO_2} and CO_{2_p}. All values were within the normal range, although the values based on pH and CO_{2_p} tended to be slightly more acidotic than those based on direct P_{CO_2} and CO_{2_p}.

The estimations of P_{CO_2} are probably reliable to within 5 per cent of the observed P_{CO_2}. The random error in the estimation of buffer base approximates 1.0 mEq. per L. Oxygen saturation may have been underestimated during this study by as much as 10 per cent since the hematocrit is an unreliable index of oxygen capacity. Errors of this magnitude in estimation of oxygen saturation have an insignificant effect on the calculation of buffer base (10), and hence satisfied the requirements of this study. No other significance should be ascribed to the values for oxygen saturation reported herein.

In the following discussion the term "respiratory acidosis" will refer to any elevation of P_{CO_2} above 45 mm. Hg; "metabolic acidosis" will denote any measurable reduction of the buffer base.

RESULTS

Serial estimations of acid-base balance were obtained on 25 patients before and during anesthesia produced by nitrous oxide, cyclopropane, ethylene, thiopental and regional block anesthesia, alone and in various combinations, and for from one to four hours following the termination of anesthesia (Table II). An average of 7 complete manometric analyses was made during each study; the range was 3 to 15. An average of 4 manometric analyses was obtained during anesthesia; the range was 2 to 8. An average of 43 measurements of arterial blood pH was made during each study.

The average duration of anesthesia was 191 minutes. The surgical operations were representative of a variety of major and minor procedures, including 5 intrapleural procedures (Table II). Anesthesia was prolonged because of the current study in a number of minor operations, such as uterine curettage.

Elevations of CO_2 tension exceeding 10 mm. Hg were observed in 18 subjects, of which 15 exhibited reductions of buffer base exceeding 3 millequivalents per liter of blood. Table III summarizes the measurements obtained on a representative subject from this group. R. S. had an essentially normal CO_2 tension before induction of anesthesia, but exhibited a mild degree of metabolic acidosis as evidenced by a buffer base of 45 mEq. per L. Following induction of anesthesia the pH fell and the P_{CO_2} became elevated to 55 mm. Hg. Although the P_{CO_2} increased to 75 mm. Hg, the plasma CO_2 content remained constant within 1 mM per L. This is expressed as a sharp initial reduction of the buffer base and further progressive reduction totalling 6.6 mEq. per L. Within one-half hour following the termination of anesthesia, the P_{CO_2} had returned to 50 mm. Hg and the metabolic acidosis had begun to resolve.

The greatest changes of P_{CO_2} and buffer base encountered in these 25 consecutively-studied procedures, during which anesthesia was produced by agents other than diethyl ether, are presented in Table IV. Data obtained during ether anesthesia are not included since it has been shown (12, 13)

TABLE II

A summary of the number of measurements carried out on anesthetized patients *

Subject	Anesthesia	Duration of anesthesia	Operation	Number of manometric analyses		Number of pH measurements	Oxygen saturation		
				During anesthesia	Total		Before	During	End
		min.					%	%	%
H. W.	Cyclopropane	127	D & C	8	15	42		92	
J. R.	Peridural	140	Prostatectomy	2	4	21	79	79	
P. B.	Thiopental-N_2O	133	Midthigh amputation	3	3	21	86	78	
T. F.	Thiopental-N_2O	165	Herniorrhaphy	2	5	40	78	79	78
O. T.	Cyclopropane	120	D & C	4	7	53	90	100	100
I. C.	Thiopental-N_2O	300	Vaginal plasty and hysterectomy	4	7	55	100	97	95
M. L.	Thiopental-N_2O-curare	225	Gastrectomy	3	5	18	82	84	
C. E.	Cyclopropane	155	D & C	4	7	40	93	100	96
R. T.	Cyclopropane	170	Ankle fusion	11	15	40			
A. S.	Epidural-meperidine-thiopental	270	Cholecystectomy	4	7	39	87	79	74
E. T.	Cyclopropane	375	Gastrectomy	4	6	53	72	79	79
M. S.	Cyclopropane	140	Closure gastrostomy	2	6	29	73	79	79
E. J.	Cyclopropane	135	Herniorrhaphy	3	6	28	82	90	90
S. E.	Cyclopropane	180	Herniorrhaphy	5	8	44	90	96	94
J. E.	Cyclopropane	130	D & C	4	8	33	85	96	85
G. R.	Cyclopropane	165	D & C	3	7	41	81	92	85
H. C.	Cyclopropane	112	Herniorrhaphy	3	6	22	84	89	98
J. V.	Cyclopropane	155	Thoracotomy	3	6	72	85	96.4	100
E. C.	Cyclopropane-curare	123	Exploratory laporotomy	3	6	55	78	83	83
R. S.	Cyclopropane	165	D & C	5	7	37	86	84	89
J. B.	Cyclopropane	160	Thoracotomy	3	8	59	88	88	90
R. D.	Cyclopropane-thiopental	210	Segmental resection	5	7	43	85	84	82
G. H.	Cyclopropane	285	Ligation of ductus arteriosis	4	7	63	77	89	80
B. D.	Thiopental-N_2O-cyclopropane	335	Pneumonectomy	5	7	75	82	94	89
L. W.	Cyclopropane	290	Gastrectomy	4	8	50	72	83	80
Averages		191		4.1	7.1	42.9	83.3	87.7	86.8

* In the last three columns summarizing oxygen saturation the words "before," "during," and "end" have the same time significance as described in the footnote to Table IV.

that ether induces a metabolic acidosis of variable degree, depending on the subject, which is independent of changes in P_{CO_2}. The cases are arranged in order of ascending magnitude of P_{CO_2} elevation. The greatest depressions of buffer base coincided with the greatest elevations of P_{CO_2}. This relationship is indicated in Figure 1.

The regression equation describing the best-

TABLE III

Measurements obtained on a representative subject *†

Time	pH	CO_{2_p}	O_{2_b}	Ht	Sat.	P_{CO_2}	BB	ΔBB
10:53	7.35	25.1	14.1	35.4	86.3	43.1	45.2	
10:55	Induction of anesthesia							
11:26	7.24	25.6	15.5	36.4	91.3	55.6	42.4	−2.8
12:04	7.22	24.9	14.6	36.0	87.3	56.6	41.0	−4.2
12:23	7.18	25.1	15.1	35.5	92.1	62.1	40.8	−4.4
1:09	7.10	25.6	14.4	36.8	83.8	74.9	38.6	−6.6
1:42	7.16	25.8	14.5	35.2	89.8	66.2	40.2	−5.0
1:42	End of operation and anesthesia							
2:14	7.26	24.1	13.4	36.0	80.3	50.2	41.5	−3.7

* R. S., November 5, 1952, Cyclopropane, Dilatation and Curettage.

† pH values represent the average of at least two independent measurements made within two minutes. Additional measurements of pH between the indicated times have been omitted from the table. The changes of buffer base, with respect to the control value obtained before induction of anesthesia, are listed in the last column.

TABLE IV

*Measurements obtained on the subjects listed in Table II**

Subject	pH			CO_2 p			P_{CO_2}			BB			ΔP_{CO_2}	ΔBB
	Before	During	End	Before	During	End	Before	During	End	Before	During	End		
H. W.	7.33	7.42	7.31	23.1	20.0	21.7	41.3	29.2	40.5	43.2	42.8	41.6	−14.0	−0.4
J. R.	7.32	7.33		32.8	29.6		59.9	53.0		50.6	48.4		− 6.9	−2.2
P. B.	7.32	7.31		23.9	21.3		43.8	39.8		43.5	41.5		− 4.0	−2.0
T. F.	7.29	7.26	→	25.0	23.6	→	48.8	49.4	→	43.0	41.8	→	+ 0.6	−1.2
O. T.	7.34	7.29	→	27.4	26.5	→	47.9	51.5	→	46.2	44.8	→	+ 3.6	−1.4
I. C.	7.34	7.25	7.34	26.0	23.7	24.3	45.6	50.4	42.5	45.5	41.8	44.2	+ 5.4	−3.7
M. L.	7.28	7.22		26.5	26.6		52.9	60.2		45.1	42.3		+ 7.3	−2.8
C. E.	7.28	7.20	7.31	26.1	26.3	23.7	52.1	62.1	44.3	44.6	42.5	43.7	+10.1	−2.1
R. T.	7.33	7.20	7.38	25.4	23.4	22.4	45.2	55.5	35.8	45.3	40.5	42.5	+10.3	−4.8
A. S.	7.34	7.21	7.26	27.2	25.3		47.7	58.7	51.	46.6	41.5	42.5	+11.3	−5.1
E. T.	7.32	7.17	7.22	21.6	20.8	20.1	39.4	53.4	45.8	41.9	38.3	39.9	+14.0	−3.6
M. S.	7.35	7.23	7.33	25.6	26.0	23.7	43.8	58.0	42.3	46.2	43.1	43.5	+14.2	−3.1
E. J.	7.36	7.25	7.26	28.2	29.1	27.4	47.0	61.8	57.3	47.5	45.5	44.5	+14.8	−2.0
S. E.	7.30	7.18	7.30	27.8	27.7	26.1	53.1	68.6	49.9	46.5	43.1	45.6	+15.5	−3.4
J. E.	7.38	7.20	7.28	25.0	23.8	24.3	40.2	56.3	48.5	44.2	39.8	41.7	+16.1	−4.4
G. R.	7.31	7.14	7.17	23.5	22.8	22.3	43.7	61.4	56.3	42.8	37.8	38.4	+17.4	−5.0
H. C.	7.35	7.20	7.28	27.4	30.6	28.6	46.9	72.4	56.9	47.0	45.7	46.1	+25.0	−1.3
J. V.	7.35	7.16	7.26	27.7	30.2	27.5	47.6	77.5	57.3	47.4	41.1	44.8	+29.9	−6.3
E. C.	7.29	7.11	→	24.8	27.6	→	48.4	79.3	→	43.7	39.2	→	+30.9	−4.5
R. S.	7.35	7.10	7.16	25.1	25.6	25.8	43.1	74.9	66.2	45.2	38.6	40.2	+31.9	−6.6
J. B.	7.32	7.07	7.17	27.9	28.0	22.9	51.0	87.0	57.1	47.1	39.6	39.5	+36.0	−7.5
R. D.	7.37	7.13	7.16	26.8	29.7	32.2	43.9	80.5	83.	46.4	42.3	45.5	+36.6	−4.1
G. H.	7.33	7.10	7.23	23.9	27.4	22.2	42.8	81.5	49.3	44.2	40.1	40.8	+38.3	−4.1
B. D.	7.35	7.03	7.27	24.2	28.8	19.9	41.3	92.5	40.6	44.3	39.2	40.6	+51.2	−5.1
L. W.	7.29	6.99	7.19	23.4	29.0	25.2	45.7	106.5	61.4	42.1	36.6	41.1	+60.8	−5.5
Average	7.328	7.190†	7.252‡	25.7	26.1	24.6	45.5	64.9†	53.0†	45.2	41.5†	42.4†	+18.2	−3.69
S.D.	±0.028	±.091	±.068	±2.26	±3.43	±2.91	±4.70	±17.2	±11.8	±2.0	±2.7	±2.3	±17.9	±1.84

* The values in the columns designated "before" were obtained before induction of anesthesia. The values in the columns designated "during" were obtained from that sample of blood drawn during anesthesia at the time when the P_{CO_2} was most divergent from the control value. The columns designated "end" include values obtained within 15 minutes of the termination of anesthesia. The figures followed by an arrow indicate that the most divergent P_{CO_2} occurred near the end of anesthesia. The greatest changes of P_{CO_2} and BB, with respect to the control values, are listed in the last two columns.

† The probability, "p," according to the "t" test, that the difference between the indicated figure and the control value could be due to chance alone is less than 0.01.

‡ "p" is less than 0.02 but greater than 0.01.

fitting straight line for the points in Figure 1 is: BB = 2.4 mEq. per L. − 0.067 (ΔP_{CO_2}) ± 0.015 (ΔP_{CO_2}) in which − 2.4 mEq. per L. is the change of buffer base expected during an anesthesia conducted without observed deviation of arterial P_{CO_2}; − 0.067 mEq. per L. is the change of buffer base expected per unit increase of P_{CO_2}; and 0.015 is the standard error of the latter term. The correlation coefficient for these data is − 0.65 ± 0.11.

The time course of the change of buffer base following a sudden elevation of P_{CO_2} is illustrated by a representative case summarized in Figure 2. Three features are typical of this response: 1) a decrease in buffer base in the first blood sample drawn after depression of the pH; 2) a tendency for full development of the metabolic acidosis to lag behind the elevation of P_{CO_2}; 3) a slow return of the buffer base toward normal following improvement of the ventilation and reestablishment of a more normal P_{CO_2}. In the instance illustrated, the ventilation was not improved until anesthesia was terminated, and the resolution of the metabolic acidosis occurred in the early post-operative period. However, the disappearance of the metabolic acidosis is not dependent on termination of anesthesia; if a respiratory acidosis occurs during anesthesia and is corrected during anesthesia, the depressed buffer base may be seen to rise during anesthesia.

The data of the case illustrated by Figure 2 are replotted in Figure 3, using plasma bicarbonate content and pH as coordinates, to emphasize the failure of HCO_{3p}^- to increase following increase in P_{CO_2} in accordance with the normal buffer line of blood (refer to "The ABC of Acid-Base Chemistry" by Davenport [14] for a detailed treatment of this graph form). The observed curve follows an almost horizontal course during development of acidosis, indicating the rapidity of development of the metabolic acidosis. On the other hand, as a consequence of the slower restitution of the metabolic acidosis, the curve parallels the expected course when the P_{CO_2} is returned toward normal rapidly.

Figure 4, which summarizes the averages of the extreme values of BB and P_{CO_2} observed in 57 consecutive studies, indicates the magnitude of change to be expected as a function of: the period of anesthesia and operation, the type of anesthetic

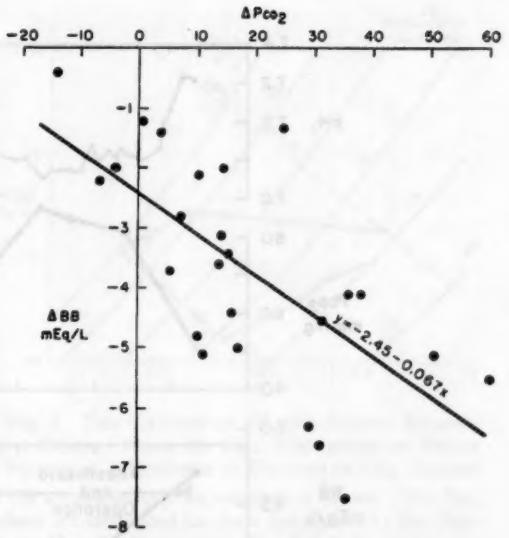

FIG. 1. CORRELATION OF THE CHANGES IN BUFFER BASE (ΔBB) WITH THE CHANGES IN ARTERIAL PLASMA CARBON DIOXIDE TENSION (ΔP_{CO_2})

See Table IV for details. The solid line represents the best-fitting straight line obtained by the method of least squares. The correlation coefficient for these data is − 0.65.

agent used, and the type of surgical operation performed. These data suggest that surgical patients tend to exhibit a mild metabolic acidosis before anesthesia and operation. The average control buffer base for all groups was 45.5 mEq. per L. compared to the mean "normal" value which is reported to be 49 mEq. per L. (10) (see also Table I). A respiratory acidosis occurred in all groups during the induction period, and was greatest in those patients subjected to thoracotomy, presumably because the incidence of endotracheal intubation was highest in these groups and the period of hypoventilation was most prolonged. During the induction period a moderate reduction of buffer base occurred which correlated with the elevation of P_{CO_2}, but tended to be greater in the groups receiving ether. Following the production of surgical pneumothorax most patients developed relatively severe respiratory acidosis. Ventilation was maintained at adequate levels most frequently during ether anesthesia for non-thoracic procedures; this is consistent with the known respiratory stimulant action of ether. On the other hand

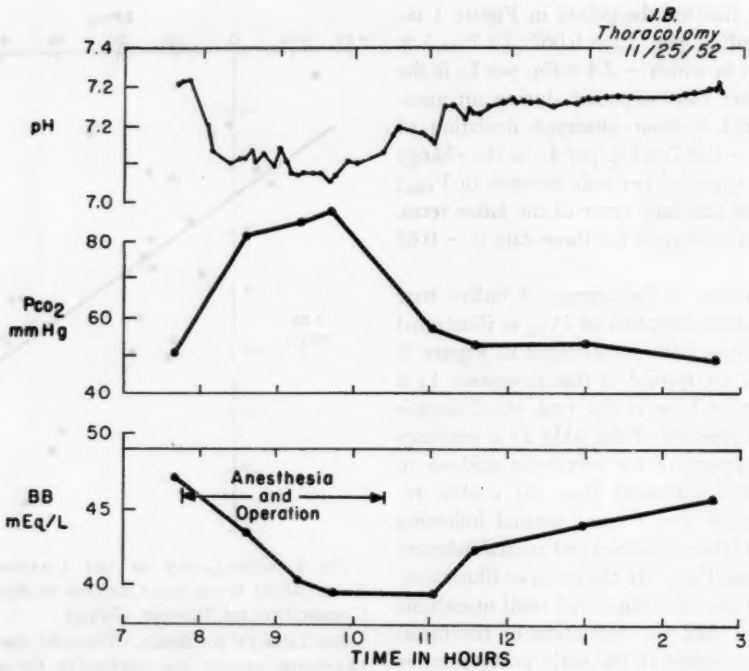

FIG. 2. THE TIME COURSE OF CHANGES OF pH, P_{CO_2}, AND BUFFER BASE DURING A REPRESENTATIVE CASE

The arrows indicate the duration of anesthesia and operation. The horizontal coordinates are adjusted to the average "normal" level.

the greatest degrees of metabolic acidosis occurred in this group during the maintenance period. In all groups the reduction of buffer base was maximum during the maintenance period. At the termination of anesthesia and operation ventilation was improved in all groups and resolution of metabolic acidosis had begun. P_{CO_2} returned to normal levels early in the recovery period. Progressive but incomplete subsidence of the metabolic acidosis was observed during the recovery period.

DISCUSSION

A metabolic acidosis occurs whenever the normal excess (approximately 49 mEq. per L.) of cations over the non-buffer anions of whole blood is reduced. A reduction of this difference can be brought about by a reduction in the total base of blood, or by an increase in the relative concentrations of any of the acids of the blood, including chloride, lactate, ketone bodies, and other highly ionized organic acids. Metabolic acidosis tends to accompany a variety of disturbances of normal physiology and is produced by a diversity of mechanisms, some of which have been extensively defined (as in the accumulation of ketone bodies during uncompensated diabetes, or the loss of sodium during severe diarrhea) and others which remain obscure.

During anesthesia a number of the known causes for production of metabolic acidosis may occur, but are normally not expected to be an obligatory accompaniment of anesthesia and operation. These causes include hemorrhagic shock, anoxemia, hepatic insufficiency, starvation, and diabetes. Ether is the only anesthetic drug among those employed during this investigation which is known to induce a metabolic acidosis directly by the accumulation of lactic acid (15, 16).

The reason why certain investigators (1, 3, 4) have observed consistently an elevation of CO_2 combining power during the first hours of respiratory acidosis, while others (2, 5) have observed

a reduction, is not immediately apparent. Anesthesia may be a factor in modifying the cellular response to respiratory acidosis by influencing cell membrane permeability or cellular enzyme reactivity. There is no evidence for the occurrence of such effects during clinical anesthesia, although they have been demonstrated *in vitro* and in unicellular preparations (17). There is, however, no consistent relationship between the use of anesthesia and the type of response observed. Metabolic alkalosis has occurred during breathing of CO_2 mixtures in unanesthetized man (4) and dog (1, 5) and in dogs anesthetized with pentobarbital (3). The same response has been obtained in the dog during respiratory acidosis resulting from morphine-induced respiratory depression (1). On the other hand, a metabolic acidosis has occurred during breathing of CO_2 mixtures in unanesthetized man and dog (5) and in the cat and dog anesthetized with barbital (2). It has also resulted from respiratory depression in anesthetized man, as reported herein, and in anesthetized dogs (Holaday, unpublished data).

Correlations based on degree and duration of respiratory acidosis are similarly unrewarding. Other factors, not yet evaluated, which might determine the type of response include pre-existing differences in hormonal or fluid and electrolyte balances, differences in regional blood flows, and degrees of muscular activity during (or in response to) respiratory acidosis. That activity of skeletal muscle during respiratory acidosis may be a significant factor is indicated by the following two studies in which a reduction of CO_2 capacity occurred in paralyzed subjects. Altschule and Sulzbach (18) present values of blood pH and CO_2 content, obtained during CO_2 administration to curarized patients, which are consistent with a rapidly developing metabolic acidosis. The descriptions of the changes of blood pH and alveolar gas CO_2 concentration in paralyzed animals during and after diffusion respiration also fit this concept (19). This factor might explain the commonly observed occurrence of severe metabolic acidosis during surgical operations in which interference with normal respiration was encountered (20–23) and the failure to observe it where respiration was known to have been adequate (12).

Failure to obtain a degree of correlation between maximum elevation of P_{CO_2} and depression of buffer base greater than that observed in Figure 4 cannot be construed as evidence against a causal relationship since the degree and duration of the respiratory acidosis were uncontrolled and the periodicity of blood sampling was not correlated with the occurrence of peak P_{CO_2} changes to establish these quantitatively in every case. Thus the opportunity for obtaining a rigorous "dose-response" relationship was not available during this study. Further studies, including controlled schedules of P_{CO_2} elevation and measurement of the distribution of the significant contributing electrolytes in the several fluid compartments of the body, will be required to define this response more thoroughly and to obtain information concerning its mechanism of production.

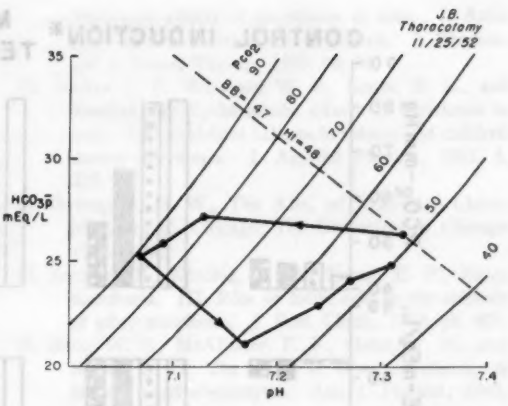

FIG. 3. THE CHANGES OF pH AND PLASMA BICARBONATE CONTENT FROM THE CASE SUMMARIZED IN FIGURE 2 PLOTTED AS A FUNCTION OF CHANGES IN CO_2 TENSION

The arrows indicate the sequence of change. The P_{CO_2} isobars are established for these coordinates by the Henderson-Hasselbach equation. The dashed line represents the buffer line for saturated blood having a buffer base of 47 mEq. per L. and a hematocrit of 48 per cent. Changes of the P_{CO_2} of this blood *in vitro* would be expected to produce changes of pH and $HCO_3\bar{p}$ coinciding with the dashed line. The vertical displacement of a point below the dashed line is an index of the extent of metabolic acidosis incurred.

The subjects of this study exhibited mild degrees of respiratory and metabolic acidosis before induction of anesthesia. Although it was not within the scope of the study to determine the causes for this, it is possible that restriction of

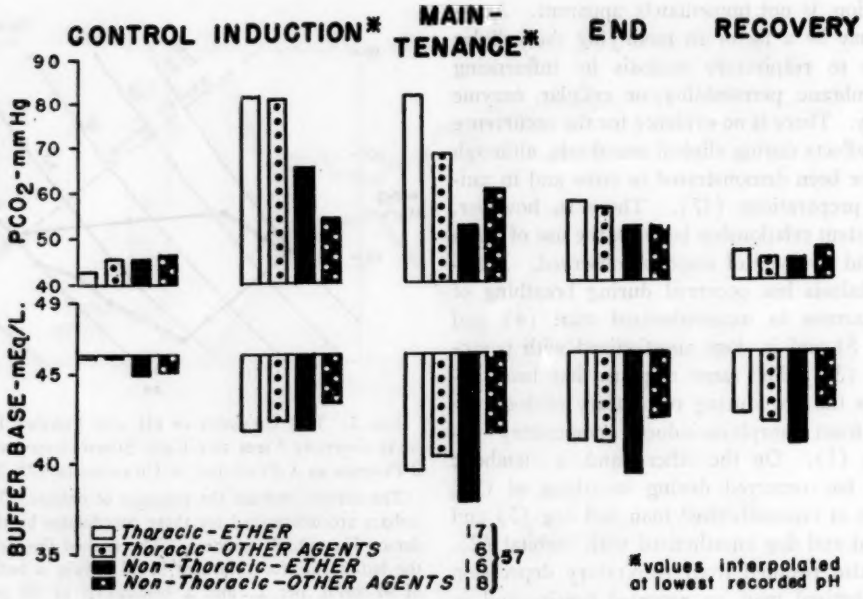

FIG. 4. SUMMARY OF STUDIES, SIMILAR TO THOSE PRESENTED IN TABLE III AND FIGURE 2, PERFORMED CONSECUTIVELY ON 57 PATIENTS

The "control" period is defined as the period immediately preceding induction of anesthesia, but usually following administration of naroctic and belladonna drugs. The "induction" period extended from the initial administration of anesthetic agent until completion of endotracheal intubation and achievement of a relatively stable level of anesthesia; the duration of this period was variable and lasted in some instances for 45 minutes. The "maintenance" period included the remainder of the operative time. The period designated "end" represents a point in time within 15 minutes before or after termination of anesthesia at which a blood sample was obtained. The values given for the "recovery" period represent averages of the last analysis obtained on those subjects who were studied for 30 minutes or more after termination of anesthesia. The average time into the recovery period during which studies were continued was 2 hours; the longest time was 6 hours. The values listed for the "induction" period and "maintenance" period were interpolated, when necessary, to the lowest pH recorded during the respective periods. The subjects are divided into 4 groups, depending upon whether or not they received ether alone or in combination with other drugs, and whether or not a thoracotomy was performed. The number of subjects included in each group is indicated in the key.

fluids and activity, preanesthetic sedation, consistent errors of determination, and disease were contributory factors.

SUMMARY AND CONCLUSIONS

The time course of alterations of acid-base balance was obtained on 25 patients before, during and after anesthesia induced with nitrous oxide, cyclopropane, ethylene, thiopental and/or regional block. The CO_2 tension of 18 subjects became elevated 10 mm. Hg or more during anesthesia. Respiratory acidosis was accompanied by a metabolic acidosis which tended to be proportional to the extent of CO_2 retention. These changes subsided rapidly following termination of anesthesia.

It is concluded that the immediate response to elevation of CO_2 tension resulting from depression of respiration in anesthetized man is a metabolic acidosis.

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AN ADAPTATION OF THE NITROUS OXIDE METHOD TO THE STUDY OF THE CEREBRAL CIRCULATION IN CHILDREN; NORMAL VALUES FOR CEREBRAL BLOOD FLOW AND CEREBRAL METABOLIC RATE IN CHILDHOOD[1]

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The nitrous oxide method for determining cerebral blood flow as originally described by Kety and Schmidt (1) has been unsuitable for use in children because of the fairly large amounts of blood withdrawn in its performance and because of its requirement of a greater degree of active cooperation than can be expected from most children under the circumstances of the procedure. Although the integrated sample modification of Scheinberg and Stead (2) permits a considerable reduction in the amount of blood drawn, it loses a valuable check on potential errors arising from defects in the respiratory system or the presence of extracerebral contamination of the internal jugular venous blood. Furthermore, timing and dilution problems associated with the Scheinberg modification lead to errors unless extensive precautions are taken (3). Recently, Baird and Garfunkel (4) have employed a modification of the original method in studies of the cerebral blood flow in infants and children. Blood requirements were reduced by the use of micro-gas analytical techniques (5, 6), but the necessary cooperation was achieved only by sedation with barbiturates. Although these drugs, in semi-narcotic doses, have been shown to be without effect on the cerebral circulation and oxygen consumption in adults (7), this has not been established in children. When anesthetic doses are employed, a marked reduction in cerebral oxygen consumption has been observed despite a slight increase in cerebral blood flow (8), the latter change probably a secondary effect of respiratory depression and consequent elevation of blood carbon dioxide tension (9). It is difficult to estimate the extent to which the Baird and Garfunkel method (4) is influenced by the sedation, inasmuch as neither the doses of barbiturates nor the values for blood carbon dioxide tension are reported.

The present communication describes a modification of the original method which is suitable for use without sedation in normal children. To test its validity it has been applied to a group of normal young men for which comparative values obtained with the original technique are available. Values are then presented for cerebral blood flow (CBF), cerebral oxygen consumption ($CMRO_2$) and cerebral vascular resistance (CVR) in normal children.

METHOD

The changes in the classical procedure were directed along two lines: 1) reduction in the amount of blood drawn, and 2) achieving active cooperation of the child by minimizing discomfort and anxiety.

Reduction in the amount of blood drawn. A micro method was developed for the determination of nitrous oxide in blood (10) which permitted the measurement of nitrous oxide content in 0.2 ml. of blood and reduced the total requirement for the estimation of cerebral blood flow to 11 ml. To permit replicate determinations, the actual volume of the blood samples was reduced only to 1 ml., but this reduction was sufficient to greatly enhance the importance of dead space in the sampling system as a source of error. Therefore, the multiple sampling manifold and the standard luer connections of the original technique were not employed. Small bore plastic tubing[4]

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[4] "Tygon" (I.D. 0.034" × O.D. 0.050") was supplied by U. S. Stoneware Co., Akron, Ohio.

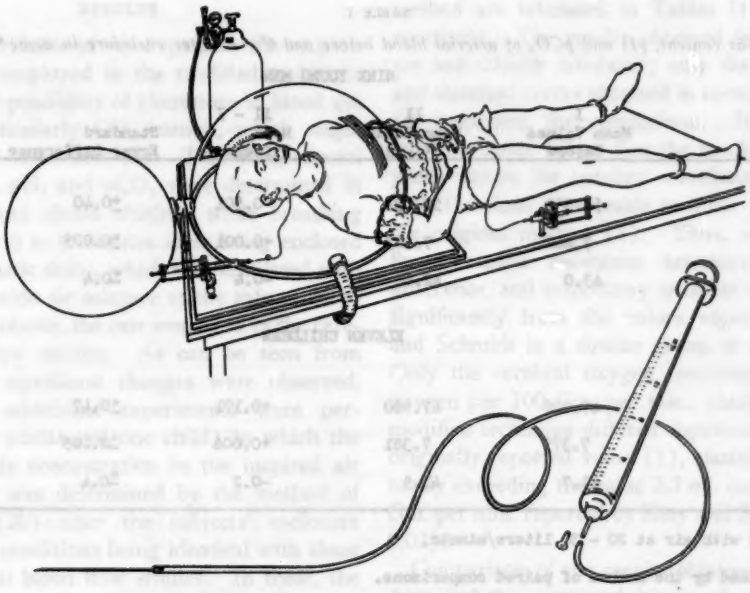

FIG. 1. APPARATUS USED IN THE DETERMINATION OF CEREBRAL BLOOD FLOW IN CHILDREN

was attached directly to hub-free needles, the other end being interchangeably connected to modified 1-ml. tuberculin syringes. Number 20 gauge stainless steel tubing, 1 cm. in length, was sealed into the glass tips of the latter, thereby reducing their dead space to approximately 0.01 ml. (Figure 1).

Achieving cooperation. To avoid the anxiety associated with the use of an anesthesia mask, a transparent acrylic plastic hemisphere, 24 inches in diameter,[5] was used to administer the nitrous oxide air mixture. This permitted the child to watch motion pictures projected upon the ceiling before and during the test period. To minimize pain in establishing the sampling systems, meticulously sharpened No. 23 gauge needles were used, the bores of which were polished[6] and coated with a silicone solution[7] to prevent clotting.

The actual procedure was as follows: After the child had become familiar with the apparatus and the details of the procedure, he was placed on a table covered with a hard mattress. The legs were immobilized with elastic bandages on a suitable board. The femoral artery puncture was performed in the usual aseptic manner using the needle with sterile tubing attached (Figure 1) after infiltrating the superficial layers of the skin with a small amount of 1 per cent procaine. The other end of the tubing was then connected to a 5-ml. syringe pipette filled with dilute heparin solution (2 mg. per ml.). After blood had been allowed to flow into the syringe to clear the system of air bubbles, heparin solution was flushed through the tubing and needle by twisting the volume control screw of the syringe pipette. Occasional quarter turns of the latter served to prevent backflow of blood into the needle. The puncture of the superior bulb of the internal jugular vein was carried out in the usual manner (11, 12) with the hub-free needle and attached plastic tubing (Figure 1). Heparin solution was introduced into the system as described for the femoral arterial puncture. The length of the needle selected varied between 1½ and 2 inches and was chosen according to the size of the child so that there was the shortest possible length protruding beyond the surface of the skin once it was properly in place. This allowed free movement of the head and neck without risking its displacement. After the sampling systems had been established and flushed with heparin solution, the dome was placed over the head and shoulders, and plastic sheeting, glued to its free edge, was tucked in tightly around the trunk. When used with adults, it was more comfortable for the subject to have arm ports provided in the hemisphere so that the hands might rest folded on the abdomen. Until the start of the test period, the dome was supported with a prop at the upper end, and an electric fan circulated air through the opened chamber.

[5] Made by Farquhar Transparent Globes, Philadelphia, Pa.

[6] This was done by using an aqueous suspension of gamma alumina with rapid alternating movements of a 3-0 nylon suture threaded through the needle bore.

[7] "Desicote" supplied by Beckman Instrument Co.

TABLE I

*Gas content, pH and pCO_2 of arterial blood before and 6' — 8' after enclosure in dome**

	I Mean Values Before	II Mean Values 6' - 8'	II - I Mean Difference	Standard Error Difference	p Value*
NINE YOUNG MEN					
CO_2 content	50.532	50.031	−0.501	±0.40	>0.2
pH	7.402	7.403	+0.001	±0.005	>0.7
pCO_2	43.0	42.6	−0.4	±0.4	>0.3
ELEVEN CHILDREN					
CO_2 content	47.808	47.980	+0.171	±0.47	>0.6
pH	7.375	7.381	+0.006	±0.005	>0.2
pCO_2	41.7	41.5	−0.2	±0.4	>0.6

* Ventilated with air at 20 - 30 liters/minute.

* As determined by the method of paired comparisons.

A weather balloon,[8] filled to 3 feet in diameter with the usual gas mixture (15 per cent nitrous oxide, 21 per cent oxygen, and 64 per cent nitrogen) and clamped at the neck with an intestinal clamp, was connected to the dome over an opening 1½ inches in diameter. After withdrawal of a blood sample to be used as the blank for the nitrous oxide analyses, the dome was lowered, and the balloon clamp released to permit a rapid washout of the air in the dome. Simultaneously, drawing of the integrated first minute samples of arterial and cerebral venous blood was begun, the rate being 0.25 ml. every fifteen seconds. Before the balloon had become completely deflated, a tank containing the gas mixture, and connected to the dome by a rubber hose, was turned on at the rate of 20 to 30 liters per min. The number and timing of subsequent samples were the same as in the original method except that each was taken over a somewhat shorter interval, *i.e.*, 10 to 15 seconds. Before the withdrawal of each blood sample, the tubing was first filled with fresh blood by drawing back the barrel of the heparin containing syringe. All sample syringes were capped and kept in ice water until analyzed. The calculation of cerebral blood flow was done as originally described after plotting the nitrous oxide concentrations of the arterial and jugular venous blood against time (1).

In the studies reported here, in which the above procedure was used, estimations of cerebral oxygen consumption, cerebral respiratory quotient, and blood carbon dioxide tension were calculated in the usual manner and required the measurement of the appropriate blood constituents. Blood oxygen and carbon dioxide contents were determined by the manometric technique of Van Slyke and Neill (13) as modified by Kety (14). Hemoglobin was estimated as cyanmethemoglobin (15) in a Klett-Summerson photometer using the copper sulfate standard (16). Blood pH was measured at ambient temperature by means of a Cambridge pH meter and micro glass electrode and corrected to 37° C. by the factors of Rosenthal (17). Blood carbon dioxide tension was computed by means of the nomogram of Peters and Van Slyke (18).

The modified method for CBF determination was tested on a group of normal, young male university students drawn from the same population previously studied by one of us (19). The children studied by this technique included five boys and four girls between the ages of 3 and 11 years. This was a highly selected group of the most cooperative children. Thirty-five others were originally selected for the procedure, but at one stage or another in the preparation (usually just before the needle punctures) became uncooperative. The two 3-year-old children required some restraint at the time of the needle punctures, but thereafter one went to sleep and the other was content to lie quietly. The past medical history of the children was negative for central nervous system disease, except for occasional headaches in two (J.H. and R.G.), and two febrile convulsions in one (M.C.). The latter had had one such convulsion two months prior to the study but no recurrence subsequently over a three-year period. At the time of this writing she was well and free of any signs of any central nervous system disorder.

[8] Supplied by Seyfang Laboratories, Atlantic City, New Jersey.

RESULTS

The large volume of dead space in the respiratory system employed in the modified technique suggested the possibility of alterations in blood gas tensions, particularly CO_2 tension, which might alter cerebral blood flow (9). Therefore, arterial CO_2 content, pH, and pCO_2 were determined in nine adults and eleven children while breathing room air and 6 to 8 minutes after being enclosed within the plastic dome, which was ventilated with the nitrous oxide air mixture at the rate of 20 to 30 liters per minute, the rate employed in the cerebral blood flow studies. As can be seen from Table I, no significant changes were observed. Also, three additional experiments were performed (two adults and one child) in which the carbon dioxide concentration in the inspired air in the dome was determined by the method of Scholander (20) after the subjects' enclosure within it, the conditions being identical with those of the cerebral blood flow studies. In these, the carbon dioxide concentration was found to rise to 0.4, 0.5, and 0.3 per cent, respectively, none of these values approaching the 2.5 per cent level which Patterson, Heyman, Battey, and Ferguson (21) have found to be the threshold for alteration of cerebral blood flow.

Data on blood constituents and the cerebral circulatory functions obtained with the modified method are tabulated in Tables II and III, respectively. The results obtained in the children are individually tabulated; only the mean values and standard errors obtained in normal young men are presented for comparison. It is apparent from the latter values that the modified technique yields values for cerebral circulatory and metabolic functions comparable to those obtained with the original method (1). Thus, cerebral blood flow, vascular resistance, arteriovenous oxygen difference, and respiratory quotient did not differ significantly from the values reported by Kety and Schmidt in a similar group of subjects (1). Only the cerebral oxygen consumption, 4.2 ml. oxygen per 100 Gm. per min., obtained with the modified technique differed significantly from the originally reported value (1), statistically significantly exceeding the value 3.3 ml. oxygen per 100 Gm. per min. reported by Kety and Schmidt ($p < 0.02$).

Comparison of the results obtained in the children and the young adults reveals some notable differences. The differences found in pulse rate, blood pressure, hemoglobin concentration, blood gas concentrations, and blood pH were all to be expected in view of the established normal values for children (22, 23). The mean cerebral blood flow in children, 106 ml. per 100 Gm. per min., was found to be considerably greater than the mean

TABLE II

Blood constituents in children and adults using the modified technique

Subject	Age	Hgb. grams %	Oxygen Content volumes %		CO_2 Content volumes %		pH		CO_2 Tension	
			Arterial	Venous	Arterial	Venous	Arterial	Venous	Arterial	Venous
Children – 9 cases										
J.H.	3	12.6	15.57	10.40	43.20	47.12	7.35	7.31	40	46
M.C.	3	11.8	15.07	9.75	46.76	51.88	7.34	7.31	43	52
Q.J.	4	13.8	17.05	11.92	49.14	54.47	7.36	7.30	46	56
W.O.	5	11.2	14.49	10.25	46.85	50.91	7.39	7.36	38	46
M.T.	6	12.0	13.73	9.12	43.01	47.36	7.33	7.29	41	50
R.G.	6	12.7	16.16	11.03	44.66	50.39	7.45	7.40	34	42
A.K.	7	12.6	16.13	11.49	46.00	50.29	7.37	7.34	41	47
S.B.	10	11.8	15.60	11.05	49.75	54.39	7.39	7.35	42	49
T.E.	11	11.7	16.48	11.60	47.84	52.54	7.41	7.37	38	46
Mean	6.1	12.24	15.59	10.73	46.36	51.04	7.38	7.34	40.0	48.2
Stand. Error	±0.9	±0.3	±0.31	±0.31	±0.8	±0.9	±0.01	±0.01	±1.1	±1.4
Adults – 12 cases										
Mean	24.5	14.91	18.74	11.75	49.86	56.31	7.40	7.35	42.8	52.3
Stand. Error	±0.8	±0.2	±0.30	±0.39	±0.72	±0.71	±0.01	±0.01	±0.6	±0.8
p* Value	<0.001	<0.001	<0.001	<0.1>0.05	<0.001	<0.001	<0.1>0.05	>0.1	<0.1>0.05	<0.05

* Determined by the method of estimating the significance of the difference between two means.

TABLE III

Comparison of data on cerebral circulatory functions in children and adults using the modified technique

Subject	Age	Pulse Rate	MABP mm. Hg.	CBF cc./100g./min.	$CMRO_2$ cc./100g./min.	$(A-V)O_2$ Vols. %	CVR mm. Hg. cc./100g./min.	Cerebral R.Q.
Children - 9 cases								
J.H.	3	102	86	120	6.2	5.16	0.7	0.77
M.C.	3	98	74	105	5.6	5.32	0.7	0.96
Q.J.	4	104	85	102	5.3	5.15	0.8	1.03
W.O.	5	94	77	100	4.3	4.25	0.8	0.96
M.Y.	6	89	83	96	4.4	4.62	0.9	0.98
R.G.	6	114	75	112	5.7	5.13	0.7	1.12
A.K.	7	87	77	96	4.4	4.64	0.8	0.92
S.B.	10	90	92	126	5.7	4.55	0.7	1.02
T.E.	11	72	83	101	4.9	4.88	0.8	0.96
Mean	6.1	94.4	81.3	106.4	5.17	4.86	0.77	0.97
Stand. Error	±0.9	±4.3	±2.0	±3.3	±0.23	±0.12	±0.02	±0.03
Adults - 12 cases								
Mean	24.5	66.8	82.4	60.1	4.18	6.99	1.42	0.94
Stand. Error	±0.8	±1.5	±1.3	±2.6	±0.47	±0.29	±0.06	±0.06
p* Value	<0.001	<0.001	>0.5	<0.001	<0.01	<0.001	<0.001	>0.6

* Determined by the method of estimating the significance of the difference between two means.

value, 60 ml. per 100 Gm. per min., observed in the young adults ($p < 0.001$). Also significantly higher in children was the cerebral oxygen consumption, the mean value being 5.2 ml. per 100 Gm. per min. as compared to 4.2 ml. per 100 Gm. per min. in the adults ($p < 0.01$). The cerebral vascular resistance in the children was 0.8 mm. Hg per 100 Gm. per min., which was statistically significantly lower than the 1.4 mm. Hg per 100 Gm. per min. observed in young adults ($p < 0.001$). No difference in the cerebral respiratory quotient (R.Q.) between the two groups was observed.

The results of repeated determinations of CBF, $CMRO_2$ and CVR in a single child using the modified method are seen in Table IV. The differences reflect not only the errors of the method but also, no doubt, some degree of physiological variation. The variability is comparable to that reported with the original procedure (24).

DISCUSSION

The modified method for measuring cerebral blood flow was found to yield values for CBF in normal young men comparable to those of the original technique, but the calculated mean values for $CMRO_2$ were found to be significantly higher. Some evidence exists that anxiety may cause an increase in $CMRO_2$ (25), but we have no reason to suspect that there was any difference in the degree of anxiety present in the two groups of young men. The discrepancy between the two methods with respect to $CMRO_2$ remains unexplained as do also similar differences in results obtained with other variations of the original method (26). Indeed, one of us (L.S.) has recently completed a series in normal young adults studied by the original techniques without modification in which the values for CBF and $CMRO_2$ are comparable to the adult values reported here (27).

The possibility that the higher values for CBF and $CMRO_2$ found in the children may have resulted from specific conditions of the procedure must be considered. In view of the report of Schmidt and Hendrix (28) that light stimulation of the retina results in an increase in blood flow to the visual cortex in cats, the projection of motion pictures, not done for the adults, might be considered of importance in accounting for the higher CBF in the children. However, the authors of this study indicate that the increase in CBF was confined only to a small, sharply demarcated area, and, therefore, it is unlikely that, even if such an increase occurred it would have a measurable effect on over-all cerebral blood flow.

Furthermore, the studies in the children were conducted in a dimly lighted room in order that the picture, reflected from the painted ceiling, could be seen, and, therefore, there was probably no greater visual stimulation in this group than in the adults. The films chosen were of vacuous content, but of sufficient interest to hold a child's attention without inducing anxiety. It is doubtful that they were provocative of cerebration of comparable intensity to that required in solving arithmetic problems, a form of mental activity which has been shown not to be associated with measurable increases in CBF or $CMRO_2$ (29).

An additional factor which must be considered is the degree of anxiety in the children as compared with that in the adults. Great pains were taken to minimize this, and that it was not a major factor is suggested by mean pulse rate and mean arterial blood pressure readings which are not above the normal resting level for children 6 years of age (23). Also, in the child studied on four separate occasions, there was no suggestion of a decrease in his CBF and $CMRO_2$ as he became thoroughly familiar with and, therefore, presumably less anxious about the procedure. A subjective evaluation of the subjects was that the children were less anxious than the adults.

Garfunkel, Baird, and Ziegler (30) have reported lower values for CBF and $CMRO_2$ in children than obtained by us. However, most of the subjects in their series had gross evidence of neurological or mental defects. Current studies in this laboratory (31) indicate that such abnormalities are frequently accompanied by reductions in CBF and $CMRO_2$. Furthermore, the mean age of their group was 32 months as compared with 6.1 years in our series. Although differences in techniques and their use of sedation make any conclusions only tenuous, their finding of a lower $CMRO_2$ at this much younger age level is consistent with the results of *in vitro* studies in the rat (32, 33) and dog (34), where $CMRO_2$ was found to be at a lower level in the very young animal.

In view of the high total body metabolic rate in childhood (35), it is, perhaps, to be expected that the cerebral metabolic rate would also be higher than in adult life. However, if this is related to growth, it is surprising to find that $CMRO_2$ remains at a high level after the age of 6 when the brain has reached 95 per cent of its mature size (36). Furthermore, in contrast to total body metabolic rate, which decreases with advancing age throughout the pre-adolescent years, $CMRO_2$ was found to have no significant correlation either positive or negative with age between the ages of 3 and 10 years ($r = -0.21$, $p > 0.1$). Indeed, it was found to be relatively constant over that age span. Consistent with this pattern of change in $CMRO_2$ with growth are the *in vitro* measurements in the rat (32, 33, 37), which together with our *in vivo* studies constitute further evidence that the energy requirements for brain tissue are determined independently from the body as a whole (38) and that these are associated with metabolic processes not directly related to increments in brain size. Gordan, Guadagni, Picchi, and Adams (39) have suggested that the steroid hormones may be of considerable importance in the regulation of $CMRO_2$ and that our findings, together with their observations of higher value for CBF and $CMRO_2$ than normal in testicular eunuchoidism and hypopituitrism, may be related to an inhibitory action of certain of these agents which has been observed *in vitro*.

Because of the special characteristics of cerebral maturation, a consideration of the high cerebral metabolic rate in childhood leads to an intriguing corollary. For example, it can be calculated [9] that in a young child the brain may ac-

TABLE IV

Repeated determinations on one child †

Date	CBF	$CMRO_2$	CVR	R.Q.
12/52	91	4.9	-	1.10
3/53	108	6.0	0.8	1.07
11/53	98	5.1	0.8	0.92
11/54	87	5.6	0.9	1.03
Mean	96	5.4	0.8	1.03
Stand. Error	±4.6	±0.25	±0.03	±0.04

† Because this child was subject to occasional generalized seizures requiring low doses of anticonvulsant medication for control he was not included in the series of normal children.

[9] A normal five-year-old child weighing 20 kilograms has an average metabolic rate of 36 calories per hour (25). Since the caloric value of oxygen is 4.8 cal. per liter, the total body oxygen consumption is 7.5 liters per hour or 125 ml. per minute, assuming a brain weight at this age of approximately 1200 grams and a $CMRO_2$ of 5.2 ml. per 100 Gm. per min., the total cerebral oxygen consumption equals 62 ml. per min. or half the total body uptake.

count for approximately one-half of the basal total body oxygen consumption. This calculation requires the assumption that the $CMRO_2$ in children in the basal state is not significantly lower than the values reported here, but the assumption is probably justified in view of the several reports of the lack of change in cerebral metabolic rate in conditions in which total body metabolic rate is known to be altered (19, 29, 38).

SUMMARY

1. A modification of the original method for measuring cerebral blood flow has been described which is suitable for use in normal children. The results obtained with the modified procedure proved comparable to those derived using the original technique.

2. In a group of normal children the cerebral blood flow and the cerebral metabolic rate were found to be significantly higher than in normal young adults, and the cerebral vascular resistance was significantly lower.

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RESPONSE OF PULMONARY ARTERY PRESSURE AND TOTAL PULMONARY RESISTANCE OF UNTRAINED, CONVALESCENT MAN TO PROLONGED MILD STEADY STATE EXERCISE [1]

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Although many published studies relevant to the effects of exercise on the gross pulmonary hemodynamics of patients with heart disease are available in the literature, few data are to be found which deal with normal man (1–6). There have been conflicting claims that there is no increase, no *significant* increase, a consistent increase, or an occasional decrease in the pulmonary artery pressure of normal man as the result of exercise loads eventuating in oxygen uptakes up to 400 ml. per M.[2] per min. (2) and pulmonary blood flows two to three times the resting basal levels (1, 5). Moreover, comparison among the published data is difficult because of variations in the exercise posture, the duration of exercise, the work loads employed, and the timing of blood and pressure sampling, which often are varied in different subjects within the same body of data.

Slonim, Ravin, Balchum, and Dressler (6) alone have systematically studied the continuous pulmonary artery pressure variations in 5 normal subjects in the recumbent position over a 7-minute period of exercise, employing an almost uniform work rate between 784 and 840 foot-pounds per minute. These authors noted immediate increases in pulmonary systolic, diastolic and mean pressures, which usually reached a peak between the first and third minutes of exercise with an overall tendency to a slight secondary decline by the sixth minute. A steady state was not achieved.

The present report is intended to extend these findings employing a mild and uniform *external* work load of 850 foot-pounds per minute sustained over a period of 39 minutes. This mild amount of work was selected in order that steady state be attained in a reasonably uniform period of time, that it be maintained as long as possible, and that some lower limit of valid, measurable change be established for comparison in subjects with heart disease physically unable to exercise at higher work loads and achieve the steady state (7).

MATERIALS AND METHODS

Studies were conducted on 12 subjects with normal cardio-respiratory systems who had convalesced from various illnesses (bronchopneumonia, active peptic ulcer, eczematoid dermatitis), or had been admitted for investigation of minor complaints. None were anemic or febrile. All had been ambulatory for several days before the studies were conducted. On the day prior to the experiment the subjects were exercised in the supine position at a standard work rate of 850-foot pounds per minute for a period of 39 minutes. In all but three instances the steady state, as determined by repetitive determinations of the oxygen consumption (8), was attained within 10 to 12 minutes of exercise; in the remaining three, it was attained at the 12 to 14-minute period. The following morning, at an ambient temperature of 23 degrees C., cardiac catheterization was performed in the post-absorptive state with mild sedation (Pentobarbital Sodium, 0.1 Gm.), employing standard No. 7F Cournand catheters. The supine position was maintained throughout the entire study. Following initial determinations of the right atrial, ventricular, pulmonary arterial and wedge pressures, all of these being within normal limits (9), the catheter tip was uniformly positioned in the right pulmonary artery, just to the right of the sternum. An indwelling arterial needle was placed into the brachial artery for the collection of blood specimens and the recording of peripheral arterial pressures. The heart rate was measured electrocardiographically and the respiratory rate was counted by direct observation of the flutter valve in the face mask. After a period of stabilization, control records were made of the pulmonary and brachial artery pressures and the heart and respiratory rates at the beginning of each of 3 consecutive minutes. This was followed immediately by duplicate 2-minute determinations of the oxygen consumption and cardiac output employing the Fick principle, and two more recordings of pressures, heart, and respiratory rates. These constituted

[1] Supported by a grant from United Appeal, Lorain County, Ohio.

the control data. The legs were then elevated, and the feet secured to the pedals of a variable resistance bicycle ergometer (10). The entire maneuver consumed 3 to 4 minutes. A record was then made of pulmonary and brachial artery pressures and heart and respiratory rates at the beginning of each minute for 5 minutes in this stationary position, followed by exercise at a steady work rate of 850 foot-pounds per minute. The stroke radius was 18 cm., and the thighs did not exceed a maximal elevation of 80 degrees from the horizontal, minimizing the possibility of abdominal compression.

In 7 patients (Table IA) arterial pressures and heart and respiratory rates were recorded at the beginning of each consecutive minute of exercise for 39 minutes, except during the 10 to 14, 20 to 24, and 30 to 34-minute periods, when duplicate determinations were made of the oxygen consumption and the cardiac output. In 3 patients (Table IB) the identical procedure was carried out, except that an intra-arterial needle was not introduced and no blood specimens were withdrawn, since it was possible that recorded pressure levels could be affected by the short-term removal of numerous blood samples. In a third group of 2 patients (Table II), following the same procedure as with the first 7, the work load was suddenly doubled at the end of 29 minutes without interruption of exercise. Recordings of pressures and rates were made every minute until a total elapsed time of 49 minutes, except during the 40 to 44-minute period when cardiac output determinations were done. At the end of this time pressures were obtained during withdrawal of the catheter in the main pulmonary artery and the right ventricle, and the experiments were abruptly terminated. The latter experiments were done to note if any change in the previously established response pattern of the pulmonary artery pressure would occur with a sudden increment in the work load.

Pressures were transduced via P23A Statham strain gauges and recorded on the Brush Multi-channel direct-writing oscillograph.[2] The reference point for central venous pressures was 10 cm. anterior to the posterior surface of the thorax, in the fourth intercostal space. Mean pressures were derived by planimetric integration of the pressure pulses in two consecutive, technically acceptable respiratory cycles. Exhaled air was measured through a dry gas test meter, 2-minute collections were made in Douglas bags and the samples analyzed in the Pauling oxygen analyzer. All expressed values for minute ventilation and oxygen consumption were corrected for STPD. The recorded data for oxygen consumptions and cardiac outputs represent the averages of two consecutive determinations which were required to vary less than 10 per cent (8) from each other to be accepted as technically valid. Blood oxygen values were determined in duplicate spectrophotometrically (11) and were required to vary less than 0.2 volume per cent from each other. Total pulmonary and estimated systemic resistances were calculated according to standard formulae and expressed as c.g.s. units (2). Mean pulmonary artery pressure only was employed in the case of the former, and those data indicate total pulmonary and not pulmonary vascular resistance. In choosing the values of mean pressure to be employed in the numerator of the formula, the average of the two pressure determinations preceding and the three immediately following each set of oxygen consumptions was employed. This minimized the error inherent to the selection of a single pressure point.

[2] The sensitivity employed for pressure recording was 1 mm. deflection for 1 mm. Hg in the case of the pulmonary, and 1 mm. deflection for 6 mm. Hg in the case of the brachial artery pressures. Error through amplifier drift was avoided by careful checking of the zero line before and introduction of a zero line after each set of recordings.

RESULTS

The data for the first group of 7 subjects are presented in Table IA, those for the control subjects in Table IB, and those for the 2 patients who underwent more prolonged exercise with an increase in the work load in Table II. The pressure values in the tables represent the averages of the individual minute-to-minute pressures for the recumbent control and stationary leg-up positions, and the 1 to 9, 15 to 19, 25 to 29, 35 to 39-minute periods of exercise (Tables IA and IB), or 1 to 9, 15 to 19, 25 to 29, 30 to 39, and 45 to 49-minute periods (Table II). Typical exercise response patterns are shown in Figures 1, 2, and 3. Average changes for pressure and other data in the first 7 patients, expressed as percentile changes from the control data, are illustrated in Figure 4.

A steady state of oxygen consumption and cardiac output was maintained at the end of the first and second sets of exercise determinations in 11 patients (oxygen uptake only in the control subjects), the lone exception being W.A. (Table IA), in whom there was a progressive rise in the cardiac output. In patients A.V., C.Q., B.B., and L.P. (Table IA) the steady state was maintained to the end of exercise, whereas in W.A., V.P., and I.R. there was an increase in the cardiac output during the third period of exercise. The first level of exercise steady state was deliberately lost in T.P. and M.G. (Table II) since the work load was doubled. Despite these changes in oxygen consumption and cardiac output the presented values are considered reasonably valid since consecutive duplicate determinations differed less than 10 percent from each other. The *minute ventilation* and *respiratory rates* followed rather closely the

TABLE IA

Prolonged mild exercise—Work load: 850 foot-pounds

Pt. Age	Sex B.S.	Oxygen consumption ml./M.²/min. Control	10–14 min.	20–24 min.	30–34 min.	Cardiac index L./M.²/min. Control	10–14 min.	20–24 min.	30–34 min.	Minute ventilation L./M.²/min. Control	10–14 min.	20–24 min.	30–34 min.	Respiratory rate Control	Feet up	10–14 min.	20–24 min.	30–34 min.
A. V. 19	WM 1.95	142	231	233	234	4.03	5.05	4.93	4.87	3.86	4.82	5.24	5.40	13.6	13.8	16.6	16.7	17.2
W. A. 23	BM 1.82	109	202	202	212	3.06	3.90	4.14	4.41	3.86	5.30	5.36	5.55	24.0	24.0	25.3	25.4	26.0
V. P. 34	WM 1.86	114	211	207	212	2.78	4.13	4.06	4.52	3.45	6.05	5.82	6.10	16.0	18.0	22.2	23.2	23.6
C. Q. 26	BF 1.45	100	174	166	174	2.87	4.02	3.91	4.04	3.47	4.93	4.66	4.85	16.0	16.0	19.6	19.6	20.4
I. R. 37	WM 1.64	123	196	199	210	3.19	3.82	3.80	4.16	3.39	4.31	4.56	4.87	14.0	15.2	19.8	19.6	19.6
B. B. 55	WM 1.64	103	197	201	202	2.17	2.95	3.01	3.00	3.33	4.60	4.96	5.13	16.0	16.0	20.0	20.0	18.0
L. P. 48	BM 1.67	115	224	214	215	2.60	3.62	3.59	3.66	4.02	6.59	6.28	6.40	14.0	14.0	18.0	18.0	18.0

* Key: W, white; B, black; M, male; B.S., body surface in square meters. Systolic, mean, and diastolic pressures from above downward in each pressure data group. The data for respiratory rate, pulmonary and brachial artery pressure represent the averages of the points sampled for each time period indicated. Blank spaces indicate technical inability to obtain data.

changes in oxygen consumption, remaining fairly stable at the end of the first two exercise periods, and tending to increase toward the end of exercise.

Elevation of the legs to the exercise position resulted in an increase in both *pulmonary and brachial artery pressure* in almost all instances. The *pulmonary artery pressure* presented two response patterns during exercise. In most cases, as exemplified in Figure 1, there was a rapid rise which remained variably sustained for the first 5 or 6 minutes. Thereafter there ensued a gradual decline so that by the end of the exercise period the pressures were only slightly above or occasionally below the control values. This was particularly true of the systolic and mean pressures. The diastolic pressure tended to show a lesser amount of decline. The second and less frequent response, as exemplified by Figure 2 (C.Q.), showed a more gradual and lesser rise in pressure which thereafter also declined progressively. This same pattern was noted in patients L.P. (IA) and J.G. (IB). In all three of these instances, the preponderant number of the pressure points recorded during exercise fell below the average and even below the lowest single pressure recorded in the control period. In no case, however, was there a failure to record at least several single pressures in the first 9 minutes which were not greater than the highest single value in the control period.

In the two subjects in whom the exercise load

TABLE II

Initial work load: 850 foot-pounds per minute—Work load increased to 1,700

Pt. Age	Sex B.S.	Oxygen consumption ml./M.²/min. Control	10–14 min.	20–24 min.	40–44 min.	Cardiac index L./M.²/min. Control	10–14 min.	20–24 min.	40–44 min.	Minute ventilation L./M.²/min. Control	10–14 min.	20–24 min.	40–44 min.	Respiratory rate Control	Feet up	10–14 min.	20–24 min.	40–44 min.
T. P. 28	BM 1.72	109	201	206	280	2.13	3.44	3.46	4.05	3.11	5.63	5.75	6.85	12.0	12.0	16.5	16.6	20.7
M. G. 32	BF 1.90	84	191	192	287	2.30	3.40	3.48	4.00	2.60	4.92	5.13	6.68	12.0	12.0	16.4	16.8	17.6

* Key: W, white; B, black; M, male; B.S., body surface in square meters. Systolic, mean, and diastolic pressures from above downward in each pressure data group. The data for respiratory rate, pulmonary and brachial artery pressure represent the averages of the points sampled for each time period indicated.

TABLE IA—*Continued*

per minute—Total exercise time: 39 minutes *

Pulmonary arterial pressure *mm. Hg*						Brachial arterial pressure *mm. Hg*						Total pulmonary resistance *C.G.S. units*				Total peripheral resistance *C.G.S. units*			
Control	Feet up	1–9 min.	15–19 min.	25–29 min.	35–39 min.	Control	Feet up	1–9 min.	15–19 min.	25–29 min.	35–39 min.	Control	10–14 min.	20–24 min.	30–34 min.	Control	10–14 min.	20–24 min.	30–34 min.
22.2	25.1	25.9	22.3	21.4	21.1	111.0	119.9	126.5	122.6	126.4	120.0	142	133	118	105	743	695	687	698
14.0	16.0	17.3	15.1	14.1	14.0	73.2	80.9	85.9	83.0	84.0	84.0								
9.5	10.6	10.7	9.5	9.5	9.3	55.0	62.2	67.7	65.2	69.6	70.0								
15.7	19.0	21.3	19.6	19.1	17.5	121.5	132.2	131.7	137.8	141.2	138.8	178	175	164	155	1,217	1,022	1,018	980
12.4	14.3	16.4	15.6	15.4	15.2	85.0	92.6	87.1	93.4	99.9	96.4								
9.3	11.1	12.5	12.3	12.6	11.5	71.0	78.0	72.5	78.0	81.8	78.4								
18.9	19.8	22.9	19.3	18.3	16.6	95.8	106.2	99.7	95.7	98.8	100.0	198	164	151	126	1,064	729	699	662
12.8	14.4	17.5	15.1	13.9	12.9	68.8	76.8	69.8	66.9	68.2	68.5								
9.0	9.9	13.5	10.8	10.4	10.3	53.2	59.2	53.5	48.8	51.4	52.2								
14.6	15.9	14.9	14.0	12.2	12.5							184	131	110	110				
9.6	10.4	10.0	8.6	8.0	7.8														
4.8	5.9	5.5	4.7	4.2	3.8														
24.4	25.1	28.7	29.1	27.5	26.3							246	218	220	198				
16.1	16.0	17.8	17.8	16.8	16.4														
8.4	9.8	11.4	10.5	9.7	9.6														
18.5	20.4	23.9	23.1	22.3	21.8	106.8	123.1	124.0	124.1	127.8	118.2	296	274	259	253	1,828	1,467	1,501	1,442
13.2	13.8	17.3	16.4	15.8	15.4	81.4	89.6	88.6	90.5	94.1	82.6								
8.9	9.4	13.0	12.1	11.3	10.5	60.7	67.8	68.8	67.6	69.8	61.2								
18.4	18.1	16.9	14.0	13.4	12.8	138.7	141.8	137.4	132.7	132.6	134.5	180	133	120	110	1,872	1,284	1,247	1,203
9.8	10.4	10.1	9.0	8.4	7.5	101.9	103.1	97.1	93.6	92.7	91.3								
5.4	6.7	7.1	5.6	5.1	5.1	83.8	83.9	79.6	77.6	78.5	79.7								

TABLE IB

Control group—Brachial artery pressures and cardiac outputs not obtained *

Pt. Age	Sex B.S.	Oxygen consumption *ml./M.²/min.*				Minute ventilation *L./M.²/min.*				Pulmonary arterial pressure *mm. Hg*					
		Control	10–14 min.	20–24 min.	30–34 min.	Control	10–14 min.	20–24 min.	30–34 min.	Control	Feet up	1–9 min.	15–19 min.	25–29 min.	35–39 min.
J. G.	WM	110	193	195	186	3.07	4.92	5.14	5.31	13.1	13.5	12.8	13.8	12.8	12.1
23	1.80									8.7	9.8	10.1	9.8	8.7	8.6
										5.4	6.9	7.8	7.1	7.3	6.8
L. Q.	BF	110	183	180	179	4.47	4.92	6.87	5.31	11.2	14.7	17.2	18.1	16.5	16.5
30	1.34									5.2	6.7	9.2	8.7	8.5	7.9
										2.8	4.3	4.8	4.4	3.7	2.8
C. S.	WM	107	217	214	216	4.06	6.91	6.78	6.83	26.3	27.5	30.0	26.2	23.3	
68	1.58									14.6	15.7	19.0	17.3	14.7	
										11.7	13.6	14.8	14.1	11.6	

* Key: W, white; B, black; M, male; B.S., body surface in square meters. Syatolic, mean, and diastolic pressures from above downward in each pressure data group. The data for respiratory rate, pulmonary and brachial artery pressure represent the averages of the points sampled for each time period indicated.

TABLE II—*Continued*

foot-pounds at 29.5 minutes of exercise—Total exercise time: 49 minutes *

Pulmonary arterial pressure *mm. Hg*							Brachial arterial pressure *mm. Hg*							Total pulmonary resistance *C.G.S. units*				Total peripheral resistance *C.G.S. units*			
Control	Feet up	1–9 min.	15–19 min.	25–29 min.	30–39 min.	45–49 min.	Control	Feet up	1–9 min.	15–19 min.	25–29 min.	30–39 min.	45–49 min.	Control	10–14 min.	20–24 min.	40–44 min.	Control	10–14 min.	20–24 min.	40–44 min.
18.5	21.3	24.2	22.9	21.1	22.6	18.7	107.4	107.8	118.0	115.4	114.8	122.6	120.2								
10.6	12.9	14.6	12.8	12.5	13.8	11.4	80.8	83.6	87.8	88.2	83.4	90.1	88.3	231	180	169	143	1,764	1,193	1,135	1,043
7.7	8.6	10.4	8.1	8.5	9.2	7.4	72.1	71.2	77.3	74.8	73.8	78.3	77.4								
17.7	21.8	20.9	18.1	15.5	17.9	15.3	111.1	116.6	119.1	114.6	111.7	115.4	111.3								
13.2	15.1	17.2	14.8	13.2	14.2	12.0	82.2	88.2	90.7	85.4	82.5	84.2	79.1	241	188	164	127	1,504	1,085	997	857
7.5	8.4	10.5	8.3	8.2	9.0	7.2	62.7	70.0	70.0	66.7	64.9	66.6	65.2								

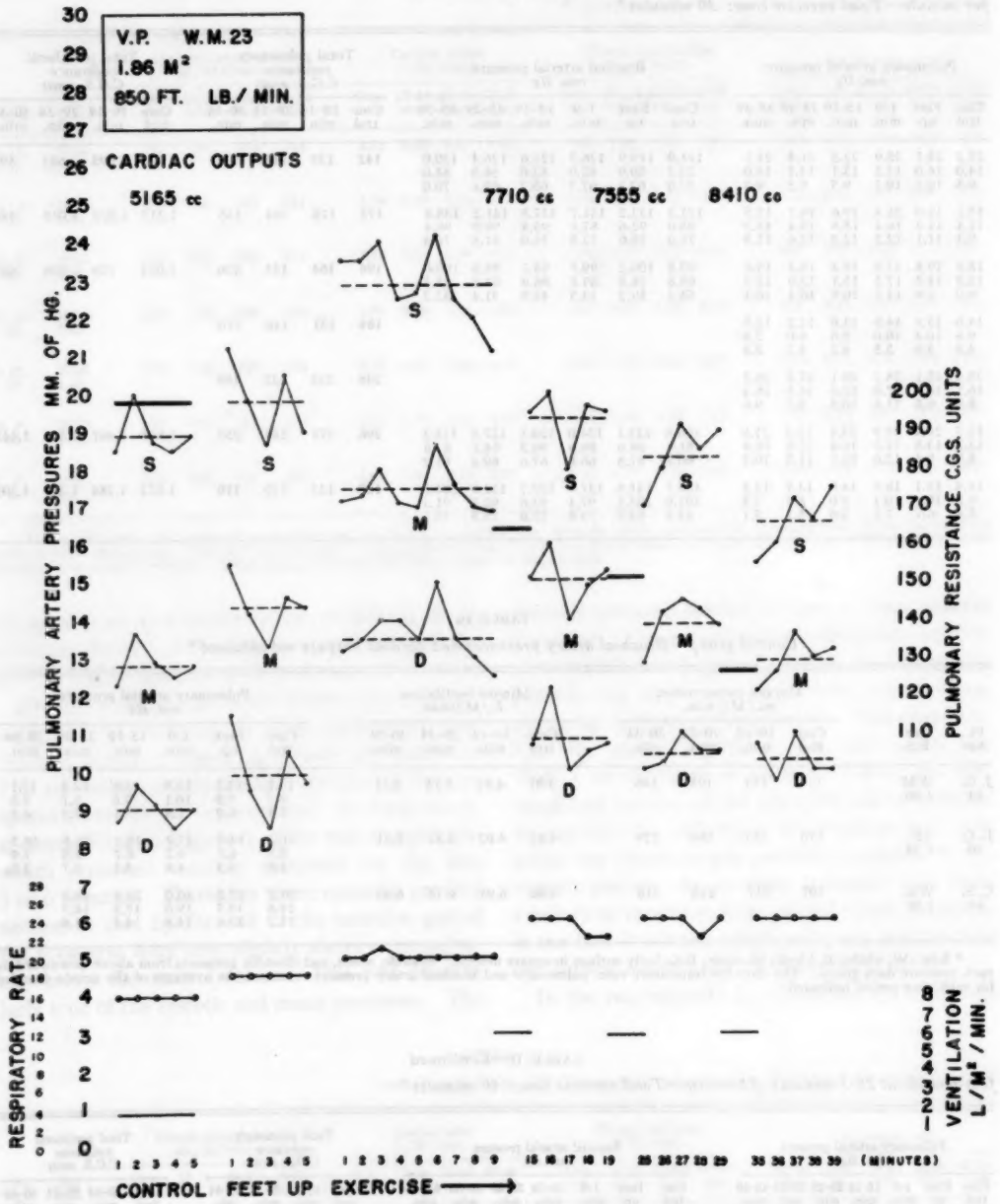

FIG. 1. RESPONSE OF PULMONARY ARTERY PRESSURES, TOTAL PULMONARY RESISTANCE (HEAVY LINES), RESPIRATORY RATE (CIRCLES) AND VENTILATION IN PATIENT V.P. (SEE TEXT)

S, M, and D = systolic, mean and diastolic pressures. The horizontal interrupted lines indicate the average value for each set of pressure points.

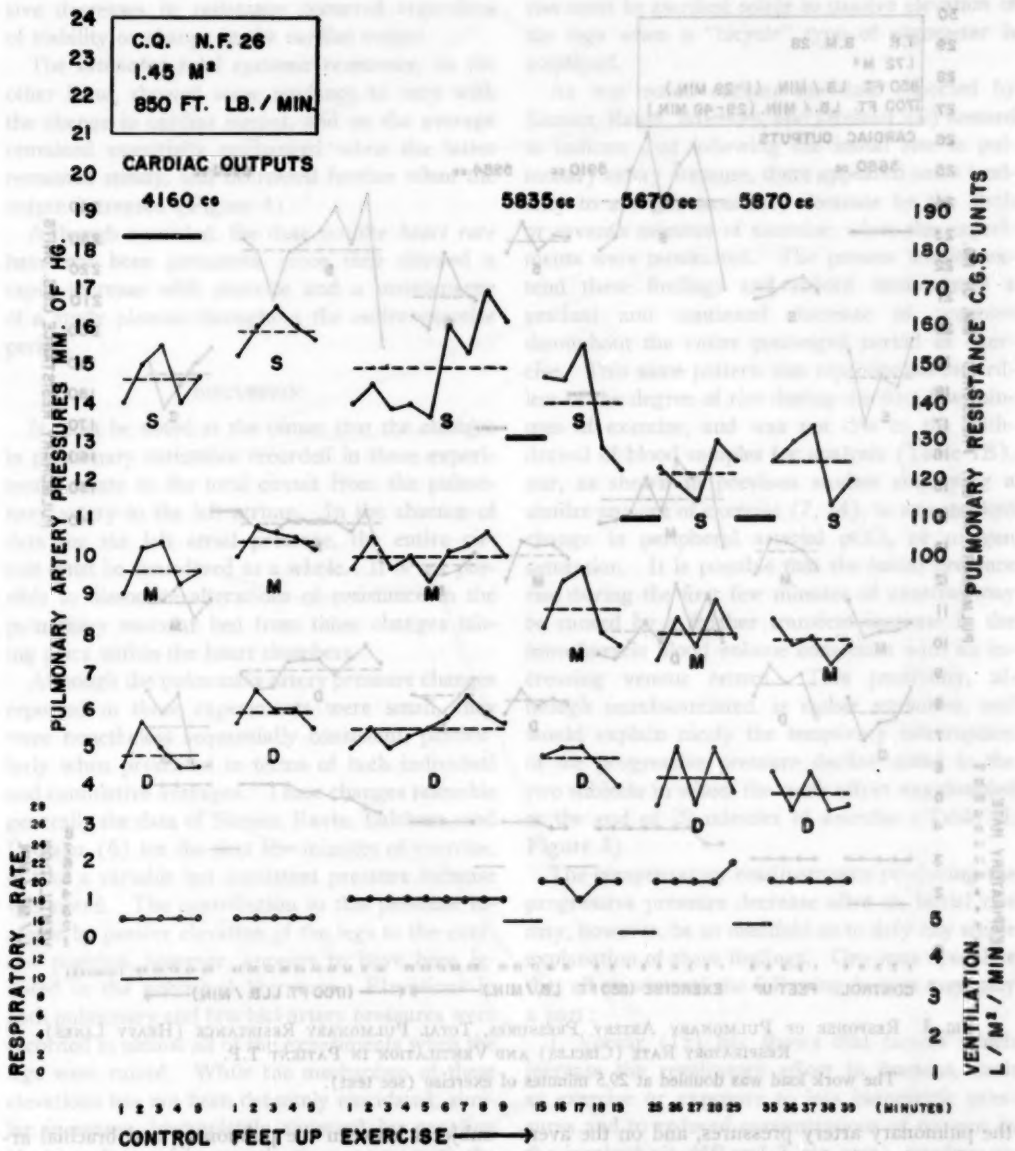

FIG. 2. RESPONSE OF PULMONARY ARTERY PRESSURES, TOTAL PULMONARY RESISTANCE (HEAVY LINES), RESPIRATORY RATE (CIRCLES) AND VENTILATION IN PATIENT C.Q. (SEE TEXT)

was doubled at the end of 29 minutes of exercise, as exemplified by patient T.P. (Figure 3), there was a secondary rise in pressure which did not, however, attain the same elevation as the rises recorded in the first 9 minutes of exercise. Thereafter, the pressures rapidly declined, and by the termination of exercise they were even lower than immediately prior to the increased work load.

The *brachial artery pressure* did not show the same repetitiveness of response to exercise as did

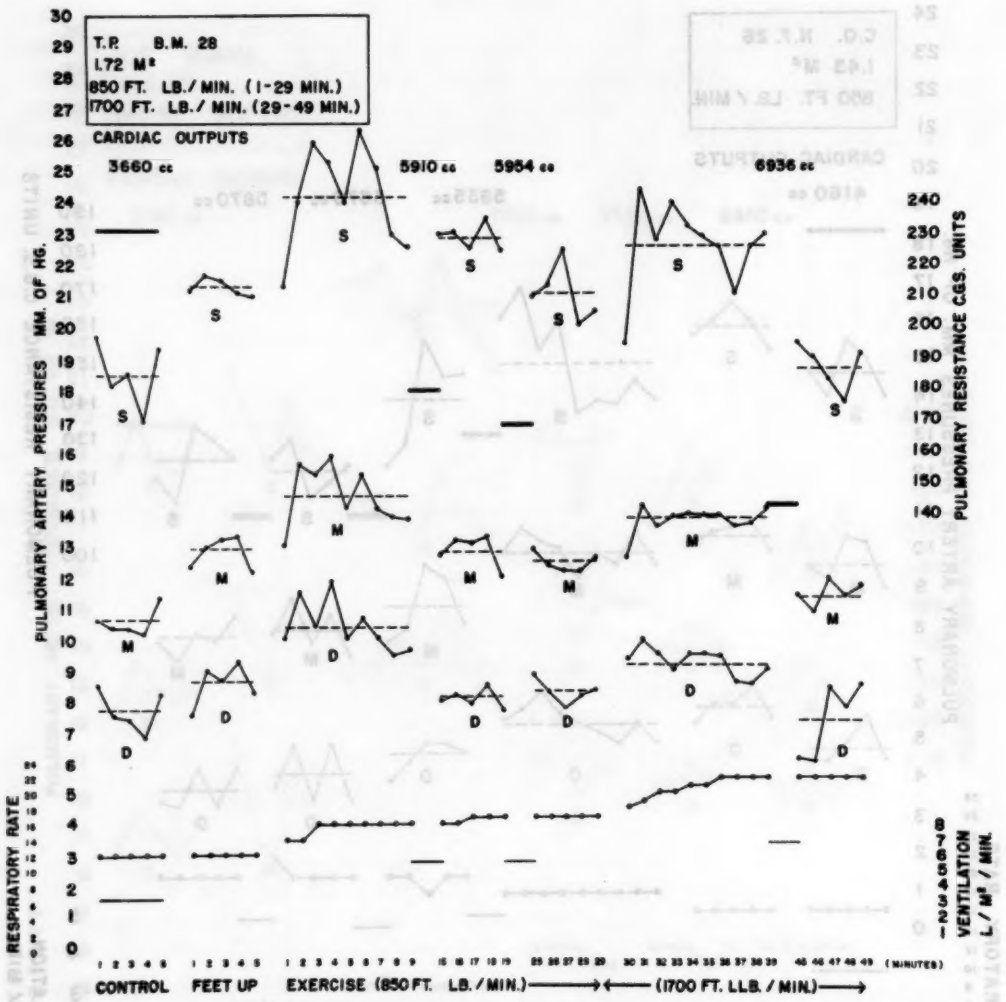

FIG. 3. RESPONSE OF PULMONARY ARTERY PRESSURES, TOTAL PULMONARY RESISTANCE (HEAVY LINES), RESPIRATORY RATE (CIRCLES) AND VENTILATION IN PATIENT T.P.

The work load was doubled at 29.5 minutes of exercise (see text).

the pulmonary artery pressures, and on the average the values during the first 9 minutes of exercise were less than those recorded in the stationary leg-up position, though higher than in the control period. Throughout the entire period of exercise the pressures tended to deviate but little from the slight elevations recorded in the period of stationary leg elevation. There was no particular correlation either on the whole or in the individual subjects between the pulmonary and brachial artery pressure changes.

The *total pulmonary resistance* showed a progressive decrease from the control values throughout the entire exercise period, except for patients I.R. and C.Q. (Table IA), in whom there was no difference between the values at the end of the first and second, and the second and third periods of exercise, respectively. Otherwise, the progres-

sive decreases in resistance occurred regardless of stability or change in the cardiac output.

The *estimated total systemic resistance*, on the other hand, showed some tendency to vary with the change in cardiac output, and on the average remained essentially unchanged when the latter remained steady, and decreased further when the output increased (Figure 4).

Although recorded, the data for the *heart rate* have not been presented, since they showed a rapid increase with exercise and a maintenance of a study plateau throughout the entire exercise period.

DISCUSSION

It must be noted at the outset that the changes in pulmonary resistance recorded in these experiments relate to the total circuit from the pulmonary artery to the left atrium. In the absence of data for the left atrial pressure, the entire circuit must be considered as a whole. It is not possible to dissociate alterations of resistance in the pulmonary vascular bed from those changes taking place within the heart chambers.

Although the pulmonary artery pressure changes reported in these experiments were small, they were nonetheless sequentially consistent, particularly when presented in terms of both individual and cumulative averages. These changes resemble generally the data of Slonim, Ravin, Balchum, and Dressler (6) for the first few minutes of exercise, in that a variable but consistent pressure increase was noted. The contribution to this pressure increase by passive elevation of the legs to the exercise position, however, appears to have been ignored in the published literature. Elevations in both pulmonary and brachial artery pressures were recorded in almost all of the experiments when the legs were raised. While the mechanism of these elevations has not been definitely elucidated, similar increases, incompletely reversed by ganglion blocking doses of hexamethonium, occur with the assumption of a 35-degree total head-down body tilt (12). It is possible that the recorded pressure increases in the pulmonary artery may be due to a translocation of blood from the lower extremities with a resultant increase in the intrathoracic blood volume (13). In any event, a portion of the pressure elevation occurring during recumbent exercise must be ascribed solely to passive elevation of the legs when a "bicycle" type of ergometer is employed.

As was noted earlier, the data reported by Slonim, Ravin, Balchum, and Dressler (6) seemed to indicate that following the initial rise in pulmonary artery pressure, there appeared some tendency to a slight secondary decrease by the sixth or seventh minutes of exercise, when the experiments were terminated. The present studies extend these findings and indeed demonstrate a gradual and continued decrease in pressure throughout the entire prolonged period of exercise. This same pattern was reproducible regardless of the degree of rise during the first few minutes of exercise, and was not due to the withdrawal of blood samples for analysis (Table IB), nor, as shown in previous studies employing a similar amount of exercise (7, 14), to any marked change in peripheral arterial pCO_2 or oxygen saturation. It is possible that the initial pressure rise during the first few minutes of exercise may be caused by a further transient increase in the intrathoracic blood volume consonant with an increasing venous return. This possibility, although unsubstantiated, is rather attractive, and would explain nicely the temporary interruption of the progressive pressure decline noted in the two subjects in whom the work effort was doubled at the end of 29 minutes of exercise (Table II, Figure 3).

The compensatory readjustments producing the progressive pressure decrease after an initial rise may, however, be so manifold as to defy any single explanation of these findings. One may speculate that all or some of the following factors may play a part:

1. Verzár (15) has shown that factors which increase the respiratory effort in humans, such as exercise or exposure to low barometric pressures and to reduced concentrations of oxygen in the inspired air (10 and 7 per cent), produce an increase of the effective residual pulmonary volume. Although there is no incontrovertible proof (15), this might be expected to produce an increased negativity of the intrapleural pressure and a consequent decrease in the intravascular pressure. This would not appear to be the sole factor involved, however, since respiratory effort ap-

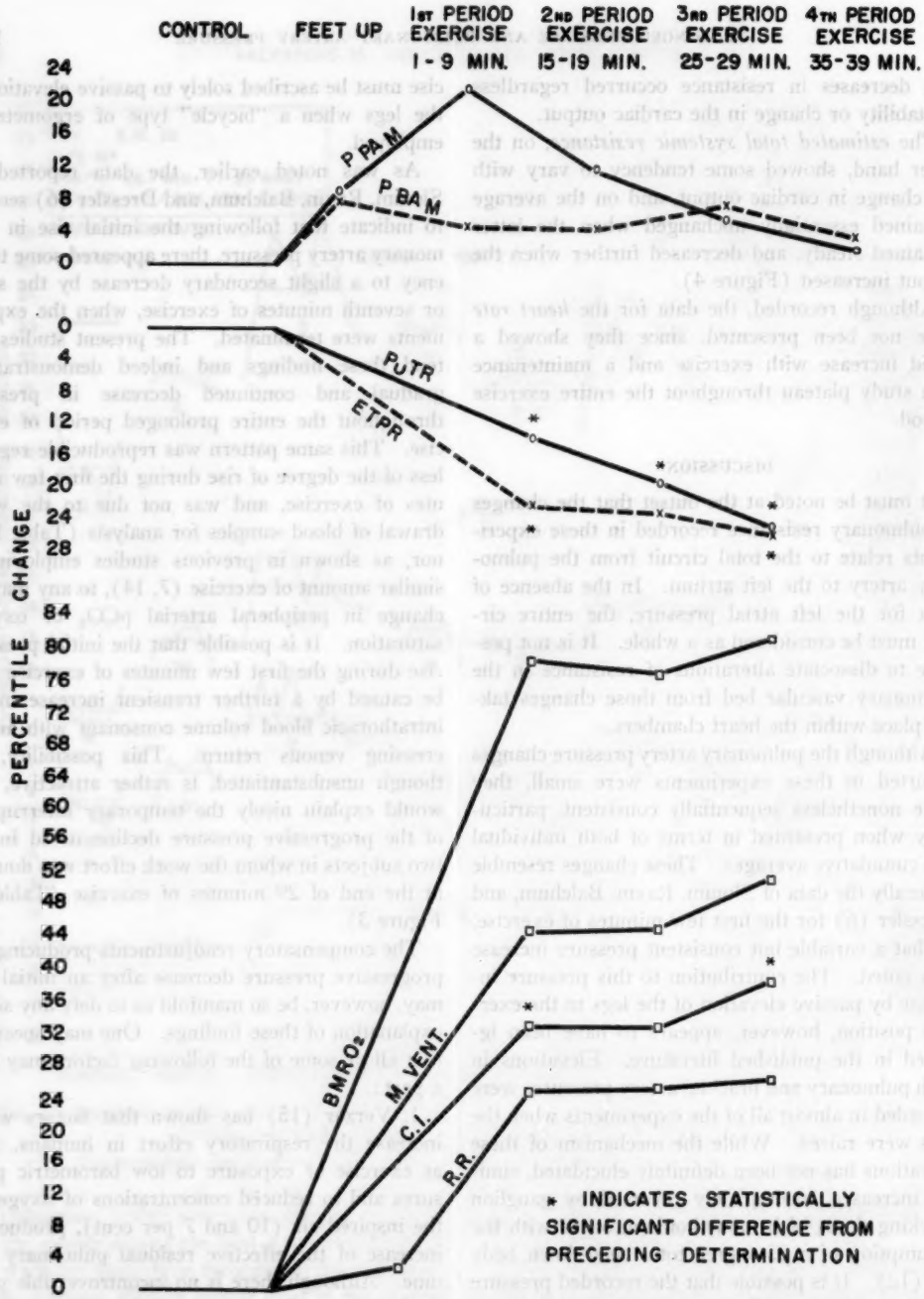

FIG. 4. AVERAGE PERCENTILE CHANGE FROM CONTROL DATA FOR PULMONARY (P_{PA_M}) AND BRACHIAL ARTERY (P_{BA_M}) MEAN PRESSURES, TOTAL PULMONARY (PU.T.R.) AND ESTIMATED TOTAL SYSTEMIC (E.T.P.R.) RESISTANCES, OXYGEN CONSUMPTION ($BMRO_2$), MINUTE VENTILATION, CARDIAC INDEX (C.I.) AND RESPIRATORY RATES (R.R.) FOR THE 7 PATIENTS IN TABLE I

Note that there is no significant difference between E.T.P.R. following the first and second exercise periods and the cardiac index for the same periods, indicating maintenance of exercise steady state for these two modalities, and an absence of steady state of Pu.T.R. (Fisher's t test, groups less than 30) throughout the entire exercise period. There is loss of the steady state for the cardiac index and the E.T.P.R. following the third period of exercise. The other data were not analyzed statistically.

peared essentially unchanged following the first and second periods of exercise, as gauged roughly by the respiratory and ventilatory rates.

2. There seems to be good evidence that in the resting basal state not all pulmonary capillaries are functional at any given moment (16). A fair amount of evidence has also accumulated that pulmonary arteriovenous anastomoses exist in mammalian (16–19) lungs. Lilienthal and Riley (16) opine that such anastomoses are not functional in the resting state, but that a relatively small stimulus may cause them to open. If this be true, and an initial increase in intrathoracic blood volume is gradually reversed (the anastomoses and capillaries meantime remaining patent, Reference 19 [3]), pulmonary artery pressure would gradually decrease although a steady state of cardiac output is maintained. It is evident from the data herein presented that a steady state of cardiac output was maintained at the end of the first and second exercise periods in 8 of 9 experiments.

3. As a result of the increased pulmonary blood flow there may occur increased pressure distention of the pulmonary vascular bed (20); this may conceivably be followed by a progressive decrease in intrinsic or neurogenically controlled vascular "tone" as a compensatory phenomenon.

4. Finally, it is not possible to assess the impression of peripheral arterial changes upon the pulmonary artery pressure, in view of the contradictory evidence relevant to the existence of pulmonary-bronchial artery anastomoses in normal human lungs (21, 22). While the absence of correlation between the peripheral and pulmonary arterial pressure changes in the present data (Figure 4) confirms the original observations of Bradford and Dean (23), there is no assurance that an interplay of forces may not occur.

Regardless of what compensatory mechanisms lead to this adaptation of the cardiopulmonary bed to an increased blood flow, it is noteworthy that total pulmonary resistance decreased progressively throughout the exercise period. While this would be expected to occur with progressive increases in the cardiac output (24) to a previously established critical limit (1, 5), it is an unexpected finding in those situations where the exercise outputs remained steady. Some or all of the mechanisms discussed above would need to be invoked. It is also of interest that the estimated total systemic resistance, on the other hand, appears to be predominantly related to the level of the cardiac output. This may be taken to indicate that specific adaptive mechanisms are operative in the cardiopulmonary bed other than would be reflected in a determination of total body vascular resistance, or that baroreceptors in the systemic circulation do not appear to bear a measurable influence on the pulmonary bed.

It is apparent that in using the term "steady state" the exact moieties to which reference is made must be defined. Thus while one may speak of "steady state" of total body oxygen consumption and cardiac output during exercise, the same concept is not applicable to pulmonary artery pressure and total pulmonary resistance within the time limits and work loads defined in these experiments.

Finally, the minute-to-minute pressure levels recorded in these studies reconcile the divergent opinions regarding the changes in pulmonary artery pressure induced by a degree of exercise insufficient to increase the pulmonary blood flow more than two or three-fold (1, 5) in normal man. The data will vary according to variability in patients and in methods used—the time of pressure sampling, the number of pressure points recorded, and, to some critical limit, the amount and duration of the imposed work load. It is suggested that just as the validity of cardiac output by the Fick method is insured by matching duplicate determinations, the accuracy of reported pressure changes should be increased by the recording of multiple points for any recorded value and a definition of the sampling time.

SUMMARY AND CONCLUSIONS

A 39-minute period of mild exercise (850 foot-pounds per minute) resulted in the maintenance of steady state, as gauged by the oxygen consumption and cardiac output, for about 23 minutes in 7 and for at least 13 minutes in 9 of 10 normal

[3] Rahn, Stroud, and Tobin showed by injection of thorotrast through a catheter lodged into a small pulmonary artery of the dog, that contrast material appeared in a pulmonary vein having bypassed the capillary bed. This bypass could be seen to continue *after* the catheter had been partially withdrawn.

subjects. In the tenth subject it was not possible to achieve a steady state of exercise response. In 2 additional subjects steady state was deliberately altered by doubling the work load after the 29th minute of exercise.

Pulmonary artery pressure rose rapidly to a small degree with the beginning of exercise, but generally began to decline after the sixth or seventh minute. The decline was progressive to the end of the allotted time for exercise, and at times pressures fell below the control resting levels. In the 2 subjects in whom the work load was doubled the decrease was temporarily interrupted by a second rise, but thereafter a pressure decline occurred once again.

Total pulmonary resistance decreased progressively throughout the entire exercise period. The decrease in resistance occurred both in those situations where the cardiac output remained unchanged after the establishment of steady exercise state, and in those in whom the cardiac output increased beyond the first established steady level.

Within the time and work load limits of the reported experiments it is suggested that whereas a reasonable measure of exercise steady state may be maintained relevant to the total body oxygen consumption and the cardiac output, the same cannot be said for the pulmonary artery pressure and the total pulmonary resistance.

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IDIOPATHIC PULMONARY HEMOSIDEROSIS. A STUDY OF THE ANEMIA AND IRON DISTRIBUTION USING RADIOIRON AND RADIOCHROMIUM [1]

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The clinical manifestations of idiopathic pulmonary hemosiderosis are well defined, though it is a rare disease. The main features are anemia in association with recurrent attacks of dyspnea, coughing with hemoptysis, cyanosis, fever, and tachycardia. Macrophages containing hemosiderin are present in the sputum. Radiographic examination of the chest during exacerbation shows diffuse homogeneous opacities or coarse, generalized, mottled densities which are usually more striking than physical examination would indicate. The acute episode usually lasts several days but may persist for several weeks. During subsidence the radiograph discloses a flecked reticular pattern which may subsequently clear completely. After a course of several years, the disease usually ends with an acute fatal exacerbation. Post mortem examination shows the lung parenchyma to be markedly engorged with blood; there is no evidence of primary heart disease. Microscopic examination reveals alveoli filled with fresh blood and macrophages containing hemosiderin, old hemorrhages, and hemosiderin granules in the alveolar walls. Analysis of lung tissue shows a marked increase of iron.

Although anemia is frequently the most prominent finding and may be severe, even in patients with little hemoptysis, the mechanism is not well defined. The anemia, when persistent, has the characteristics of iron deficiency: hypochromasia of the erythrocytes, low mean corpuscular hemoglobin content, and a low serum iron concentration. The cause of the anemia is generally regarded to be blood loss in the lungs, though a few authors have believed that the anemia is probably hemolytic in nature (1–3). The findings of reticulocytosis, normoblastemia, urobilinogenuria, and increased concentration of indirect bilirubin in serum are compatible with either intravascular destruction of erythrocytes or massive hemorrhage within the body. In the latter circumstance, hyperbilirubinemia with urobilinogenuria would be due to the resorption of blood pigment derived from erythrocyte breakdown. The results of other laboratory tests, however, are not indicative of a hemolytic mechanism. Neither increased fragility of erythrocytes, unequivocally positive direct Coombs test, nor spherocytosis, as found in acquired hemolytic anemias, has been described.

To investigate the mechanism of the anemia, we have performed studies on two patients with idiopathic pulmonary hemosiderosis during periods of remission and exacerbation. Erythropoiesis and the movement of iron within the body were studied with radioactive iron (Fe^{59}). The erythrocyte survival time was determined by the Ashby differential agglutination method in one subject and by labelling the erythrocytes with radioactive sodium chromate (Cr^{51}) in the other.

MATERIALS AND METHODS

One patient is a four-year-old Negro boy who has had signs and symptoms of the disease since the age of two. He is the subject of two previous reports (4, 5). The other patient is a twenty-nine-year-old male who developed symptoms of the disease at the age of twenty-two. He has had two periods of remission, each one lasting over two-and-a-half years. His clinical course is reported elsewhere (6). Both subjects are still living.

In the literature surveyed, forty-two cases of the disease occurred in children (1–5, 7–21), and thirteen in adults (6, 22–28).

The general procedure of the blood and tissue iron studies was modeled after the one described by Huff and his co-workers (29–31). The following sets of measurements were made with the patient in a fasting state and at bed rest:

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Blood volume determination. The total blood, erythrocyte, and plasma volumes were measured by three methods. Radioactive phosphorus (P^{32})-tagged erythrocytes, then Evans Blue dye were injected intravenously. Thirty minutes later radioiron was injected, after a sample of blood was withdrawn for the Evans Blue dye and P^{32} determinations. The details of this procedure have been reported (32). Blood samples were taken 5, 15, 30, and 45 minutes and 1, 2, 3, 6, and 24 hours after the injection of radioiron, and the radioactivity per ml. plasma determined. The plasma volume is computed by dividing the total amount of radioiron injected by the amount of radioiron present at zero time in one ml. of plasma. The total blood and erythrocyte volumes were calculated from the plasma volume and corrected hematocrit.

The determinations of the total blood, plasma, and erythrocyte volumes by the three methods were in good agreement. In the calculations, the figure used for the plasma volume was the one obtained using the Fe^{59} method; the figure for the erythrocyte volume was the one derived from the P^{32} labelling method. The number used for the total blood volume was the sum of these two figures.

Rate of plasma iron removal (turnover rate). The tracer dose of iron was given as protein-bound Fe^{59}, obtained in the following manner: A ferric ammonium citrate solution was prepared from radioactive ferric chloride by precipitating ferric hydroxide with 6 N ammonium hydroxide and heating. The precipitate was separated by centrifugation and evaporation almost to dryness. Citric acid crystals were added until the precipitate dissolved. The pH was adjusted to 7.0 with ammonium hydroxide. Isotonic saline solution was added so that the iron concentration was approximately 50 μg. per ml., and the solution was autoclaved. It was then incubated 30 minutes with fresh plasma from a normal person. The dose of Fe^{59} used was five microcuries for the adult and three microcuries for the child, contained in ten ml. of plasma. The radioiron was of high specific activity, approximately one μc. per μg. The blood obtained at the stated intervals after injection of iron was centrifuged and three ml. of plasma pipetted into a screw-top test tube for radioanalysis in a well-type NaI(Tl) scintillation counter (33). The efficiency for Fe^{59} was 16.4 per cent (364 counts per minute per 0.001 μc.); the average background was 250 counts per minute. Counting rates per ml. plasma were plotted versus time on a semilogarithmic scale. The fraction of total plasma iron removed each hour (K) was determined by dividing the natural logarithm of two by the time required for the counting rate to decrease to half of its initial value. The plasma iron turnover rate was calculated in terms of mg. per hour per liter of blood volume as follows:

$$\frac{K \times \text{plasma iron (mg. per ml.)} \times \text{plasma volume (ml.)}}{\text{Total blood volume (liters)}}$$

The plasma iron turnover is expressed in terms of mg. per hour rather than mg. per day because the plasma measurements are actually made over a period of a few hours and one can not be certain that the removal of iron from the plasma will remain fixed at this rate during a 24-hour period. The plasma iron turnover is expressed in terms of rate per liter of blood volume because we are of the opinion that blood volume is preferable to weight as a basis of comparing the plasma iron turnover (normally proportional to hemoglobin formation) of infants, children, and adults, lean or obese. The serum iron concentration was determined by the method of Kitzes, Elvehjem, and Schuette (34) on blood samples obtained just before and 4 hours after injection of radioiron. Normal values in our laboratory range between 70 and 170 μg. per 100 ml. serum.

Net incorporation of Fe^{59} in erythrocytes. The radioactivity of 3 ml. of a lysed erythrocyte solution of each blood sample drawn was determined, correcting for decay. A hematocrit was taken on each blood sample. The percentage of the administered dose in the total red cell mass was computed using the hematocrit and total erythrocyte volume as determined above.

Body distribution of Fe^{59}. Serial counting rates of radioactivity were made externally over representative volumes of blood, liver, spleen, bone marrow, and lung using a scintillation gamma ray counter. The sites used were: over the heart (for whole blood activity) at a point just medial and superior to the apex beat, over the liver in the central area of dullness at the mid-axillary line, over the spleen in the center of percussable dullness, and over the upper portion of the sacrum. These sites were marked with two per cent gentian violet. Measurements over tissue sites were made at the same time that blood samples were taken for determination of plasma and erythrocyte radioactivity. Graphs of the organ distribution of the injected radioiron were made by plotting the net counts (total counts minus body background counts) per minute per microcurie of injected Fe^{59}. All counts were corrected for Fe^{59} decay. It is well to stress that *in vitro* analysis of radioiron is not a strict quantitative determination, since the counts per minute per microcurie injected Fe^{59} vary in accordance with individual body geometry. Nevertheless, the general pattern and phase relations of an organ uptake curve is significant when compared with the one obtained in a healthy subject.

Erythrocyte survival time. The standard Ashby differential agglutination method was used to determine the life span of erythrocytes in the adult patient. Cr^{51} was used to study erythrocyte survival time in the child. The radiochromium, $Na_2Cr^{51}O_4$, had a specific activity of 1.3 mc. per mg. The patient's blood (50 ml.) was collected in a small plastic bag containing acid-citrate-dextrose solution. Forty μc. of Cr^{51} was added and the blood incubated at 37°C. for thirty minutes with occasional gentle mixing. One-half hour after the Cr^{51}-labelled blood was given, samples were drawn, and then 1, 3, 4, 7, 10, 20, 23, 27 and 56 days later. Three ml. of whole blood was hemolyzed with powdered saponin, and the net counts per minute determined in the well-type scintillation counter. Since Fe^{59}-labelled erythrocytes were also present in the circulation, Cr^{51} and Fe^{59} gamma rays were

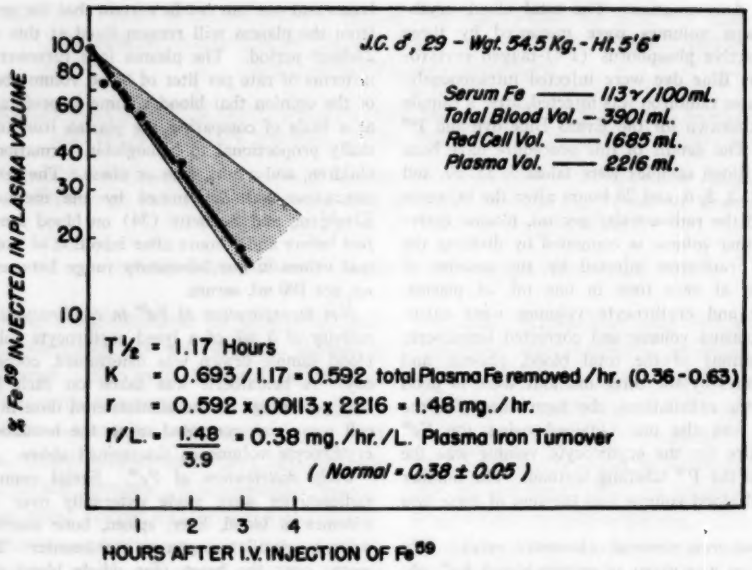
J.C. ♂, 29 - Wgt. 54.5 Kg. - Ht. 5'6"
Serum Fe —— 113γ/100ml.
Total Blood Vol. – 3901 ml.
Red Cell Vol. —— 1685 ml.
Plasma Vol. —— 2216 ml.
% Fe59 INJECTED IN PLASMA VOLUME
100
80
60
40
30
20
10
T½ = 1.17 Hours
K = 0.693 / 1.17 = 0.592 total Plasma Fe removed /hr. (0.36 - 0.63)
r = 0.592 x .00113 x 2216 = 1.48 mg./hr.
r/L. = 1.48 / 3.9 = 0.38 mg./hr./L. Plasma Iron Turnover
(Normal = 0.38 ± 0.05)
1 2 3 4
HOURS AFTER I.V. INJECTION OF Fe59

FIG. 1. PLASMA IRON REMOVAL AND TURNOVER

separated by pulse-height discrimination as previously reported (33). The efficiency of the counter for Cr^{51} was 39.1 per cent (867 counts per minute per 0.001 μc.); the average background count was 550 counts per minute. All counts were corrected for decay. The values have been expressed as a percentage of the initial Cr^{51} value obtained one-half hour (or one day if the activity at that time was higher) after the labelled erythrocyte infusion was given. The survival of erythrocyte Cr^{51} was plotted on a semilogarithmic scale.

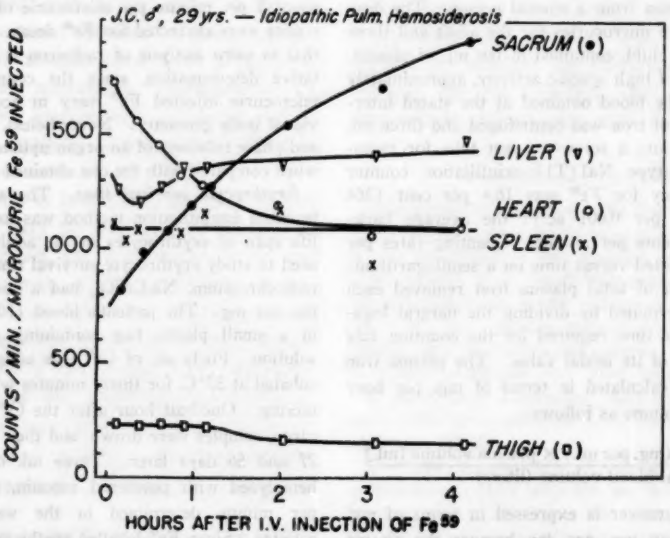

FIG. 2. BODY DISTRIBUTION OF Fe^{59} AFTER INTRAVENOUS INJECTION

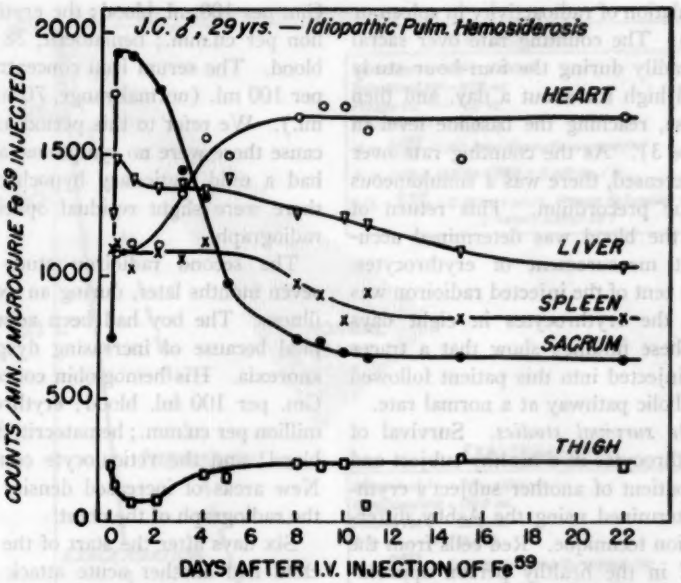

FIG. 3. BODY DISTRIBUTION OF Fe^{59} AFTER INTRAVENOUS INJECTION

RESULTS

Case 1. (*J.C.*)

This 29-year-old patient was in remission during the period of study. He had had no abnormal pulmonary signs or symptoms, and no anemia for more than one year.

A. Radioiron studies. The curves for plasma iron removal, body distribution of the injected Fe^{59}, and net incorporation of Fe^{59} in the circulating erythrocytes are shown in Figures 1, 2, 3, and 4. The results are similar to those obtained previously in twelve healthy adult men, and agree in general with the data reported by Huff and his colleagues (30, 31). The pattern was the same in healthy children (35).

In this patient, fifty per cent of the injected radioiron left the plasma in 1.17 hours. The plasma iron turnover rate was 0.38 mg. per hour per liter of blood volume (Figure 1). As radioactivity in plasma decreased there was a simultaneous increase in bone marrow. There was no ap-

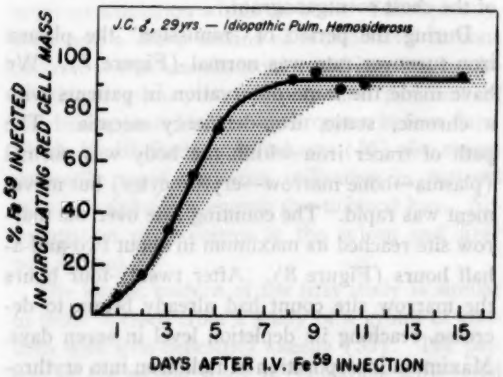

FIG. 4. NET INCORPORATION OF Fe^{59} IN ERYTHROCYTES

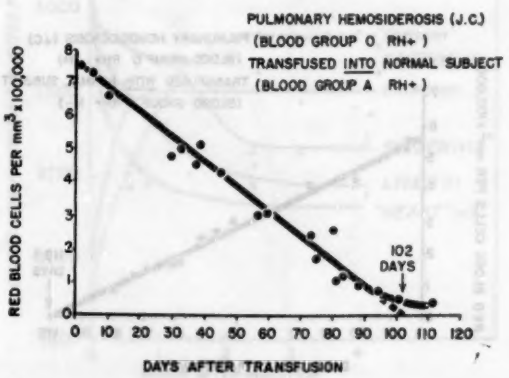

FIG. 5. ASHBY SURVIVAL OF RED BLOOD CELLS

preciable accumulation of radioactivity in spleen or liver (Figure 2). The counting rate over sacral marrow rose steadily during the four-hour study period, remained high for about a day, and then began to decrease, reaching the baseline level in ten days (Figure 3). As the counting rate over bone marrow decreased, there was a simultaneous increase over the precordium. This return of radioactivity to the blood was determined accurately by direct measurement of erythrocytes. Ninety-three per cent of the injected radioiron was incorporated in the erythrocytes in eight days (Figure 4). These findings show that a tracer amount of iron injected into this patient followed the normal metabolic pathway at a normal rate.

B. Erythrocyte survival studies. Survival of the patient's erythrocytes in a healthy subject and survival in the patient of another subject's erythrocytes were determined using the Ashby differential agglutination technique. Red cells from the patient survived in the healthy person approximately 102 days (Figure 5), and the red cells of a normal donor survived approximately 120 days in the patient's circulation (Figure 6).

Case 2. (P.M.)

Two ferrokinetic studies were performed on the four-year-old patient. The first study was begun when he was asymptomatic. Three months earlier he had suffered a mild attack of dyspnea and cough associated with a slight increase in hilar densities in the chest radiograph, but had had no pulmonary symptoms for the preceding two months. A mild hypochromic anemia had persisted. The hemoglobin concentration was 10.7 Gm. per 100 ml. blood; the erythrocytes, 4.2 million per cu.mm.; hematocrit, 38 ml. per 100 ml. blood. The serum iron concentration was 54 μg. per 100 ml. (normal range, 70 to 170 μg. per 100 ml.). We refer to this period as "remission" because there were no symptoms, although the child had a mild stationary hypochromic anemia and there were slight residual opacities in the chest radiograph.

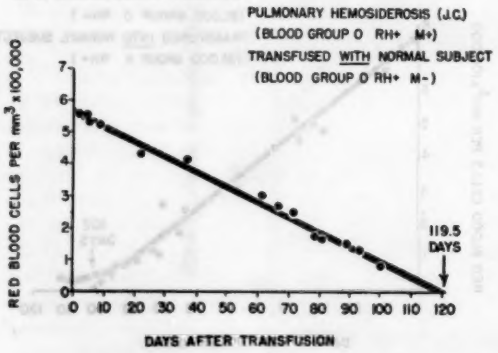

FIG. 6. ASHBY SURVIVAL OF RED BLOOD CELLS

The second radioiron study was performed seven months later, during an exacerbation of the illness. The boy had been admitted to the hospital because of increasing dyspnea, fatigue and anorexia. His hemoglobin concentration was 7.9 Gm. per 100 ml. blood; erythrocyte count, 3.45 million per cu.mm.; hematocrit, 31 ml. per 100 ml. blood; and the reticulocyte count, 9.0 per cent. New areas of increased density had appeared in the radiograph of the chest.

Six days after the start of the second study the child had another acute attack characterized by recurrent dyspnea, cough, and listlessness, with additional infiltrations in the lung fields. This occurred two days after ninety-nine per cent of the tracer iron had been incorporated in circulating erythrocytes (Figure 10). Similar mild episodes recurred during the next month. Measurements of radioactivity in the lungs and circulating erythrocytes were made periodically for 119 days.

A. Radioiron studies. The curves of the two radioiron studies are shown in Figures 7, 8, 9, and 10. Only the counting rates obtained over the anterior right lung apex are given (Figure 10), for they are representative of the other areas. The most pronounced opacities occurred in this area of the chest roentgenogram.

During the period of "remission" the plasma iron turnover rate was normal (Figure 7). We have made the same observation in patients with a chronic, static, iron-deficiency anemia. The path of tracer iron within the body was normal (plasma→bone marrow→erythrocytes) but movement was rapid. The counting rate over the marrow site reached its maximum in about two-and-a-half hours (Figure 8). After twenty-four hours the marrow site count had already begun to decrease, reaching its depletion level in seven days. Maximum incorporation of radioiron into erythrocytes occurred in seven days. There was no sig-

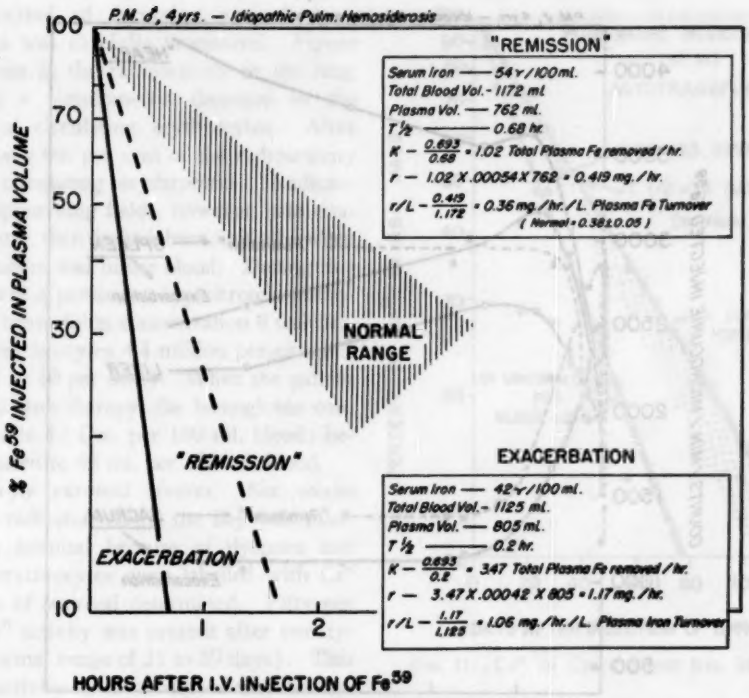
P.M. ♂, 4 yrs. — Idiopathic Pulm. Hemosiderosis
% Fe59 INJECTED IN PLASMA VOLUME
100
70
50
30
10
1
2
"REMISSION"
Serum Iron — 54 γ/100 ml.
Total Blood Vol. - 1172 ml.
Plasma Vol. — 762 ml.
T ½ — 0.68 hr.
K — 0.693/0.68 • 1.02 Total Plasma Fe removed/hr.
r — 1.02 X .00054 X 762 • 0.419 mg./hr.
r/L — 0.419/1.172 • 0.36 mg./hr./L. Plasma Fe Turnover
(Normal • 0.38±0.05)
NORMAL RANGE
"REMISSION"
EXACERBATION
EXACERBATION
Serum Iron — 42 γ/100 ml.
Total Blood Vol. - 1125 ml.
Plasma Vol. — 805 ml.
T ½ — 0.2 hr.
K — 0.693/0.2 • 3.47 Total Plasma Fe removed/hr.
r — 3.47 X .00042 X 805 • 1.17 mg./hr.
r/L — 1.17/1.125 • 1.06 mg./hr./L. Plasma Iron Turnover
HOURS AFTER I.V. INJECTION OF Fe59

FIG. 7. PLASMA IRON REMOVAL AND TURNOVER

nificant increase in radioactivity over the spleen or liver sites (Figure 9).

During the period of exacerbation the initial pattern of body distribution of radioiron was normal but its movement was extremely rapid. The rate of removal of iron from the plasma was approximately three times normal (Figure 7).

Radioiron in the bone marrow reached its maximum accumulation within an hour. Subsequently, there was a sharp decline of radioactivity in bone marrow with a simultaneous rapid increase in circulating erythrocytes. Ninety-nine per cent of the dose appeared in the erythrocytes in four days. Finch, Wolff, Rath, and Fluharty (36) also report unusually rapid radioiron utilization in patients with iron deficiency anemia due to blood loss. Accumulation of radioiron in the spleen and liver was normal.

The kinetic pattern of the first study is similar to those derived from a study of a group of children with iron-deficiency anemia (35). The pattern of the second study is compatible with rapid erythrocyte regeneration following acute blood loss superimposed upon a moderate iron deficiency anemia pattern.

In the second study, measurements of radioiron distribution in the body were continued during a

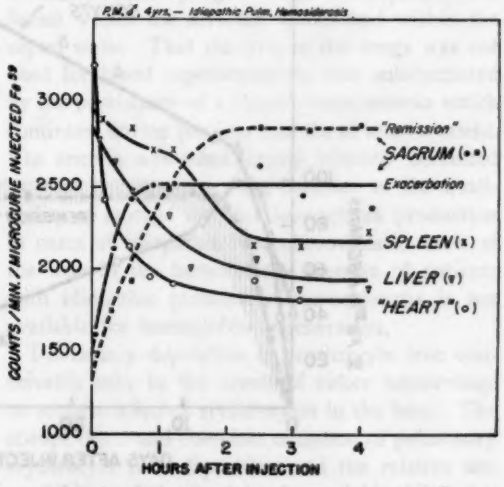

FIG. 8. BODY DISTRIBUTION OF INJECTED Fe^{59}

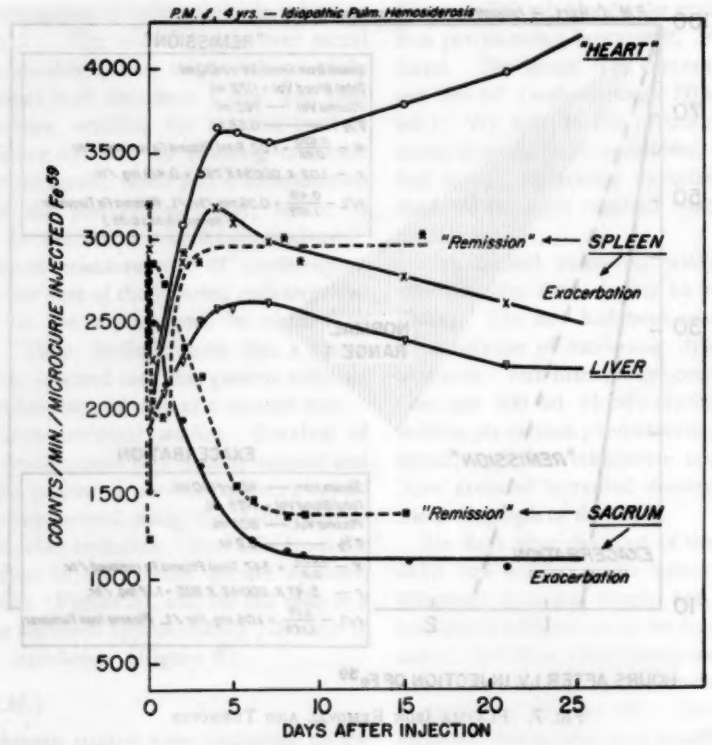

FIG. 9. BODY DISTRIBUTION OF INJECTED Fe^{59}

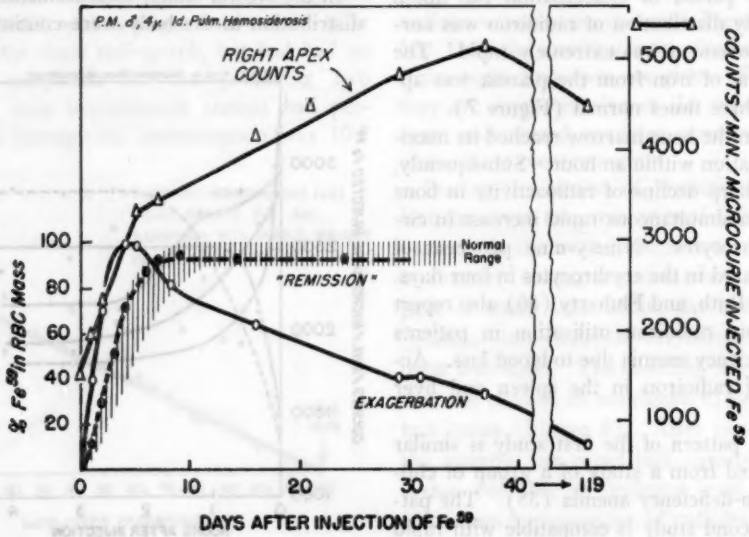

FIG. 10. NET INCORPORATION OF Fe^{59} IN ERYTHROCYTES

four-month period of recurring exacerbations. The lung area was carefully monitored. Figure 10 shows a rise in the radioactivity in the lung accompanying a simultaneous decrease in the radioactivity of circulating erythrocytes. After four months only ten per cent of the radioactivity remained in circulating erythrocytes. Radioactivity in the upper lung fields, however, was considerably greater than it had been initially when all of the radioiron was in the blood. During this period there was a persistent hypochromic microcytic anemia (hemoglobin concentration 8 to 9 Gm. per 100 ml.; erythrocytes 4.4 million per cu.mm.; reticulocytes 1 to 10 per cent). When the patient was given oral iron therapy, the hemoglobin concentration rose to 12 Gm. per 100 ml. blood; hematocrit increased to 42 ml. per 100 ml. blood.

B. Erythrocyte survival studies. Six weeks after the first radioiron study, the boy was readmitted to the hospital because of dyspnea and cough. His erythrocytes were labelled with Cr^{51} and their time of survival determined. Fifty per cent of the Cr^{51} activity was present after twenty-three days (normal range of 31 to 39 days). This reduction of activity in erythrocytes may be due to a decrease in red cell life-span or to hemorrhage. A simultaneous progressive decrease of erythrocyte radioiron indicates that hemorrhage is occurring. Fe^{59} radioactivity in circulating erythrocytes of patients with stable hemolytic anemias remains approximately constant since the radioiron of destroyed erythrocytes is rapidly re-incorporated into new erythrocytes. The radioiron in this patient's blood, however, decreased steadily during the period of study. If correction is made for the approximate amount of radioiron lost, the half-time survival of the Cr^{51}-labelled erythrocytes is within normal limits (Figure 11).

A four-day stool urobilinogen excretion study was made during the period of "remission" to determine the hemolytic index. It was 12 mg. of urobilinogen per day per 100 Gm. of hemoglobin (normal: 11 to 21).

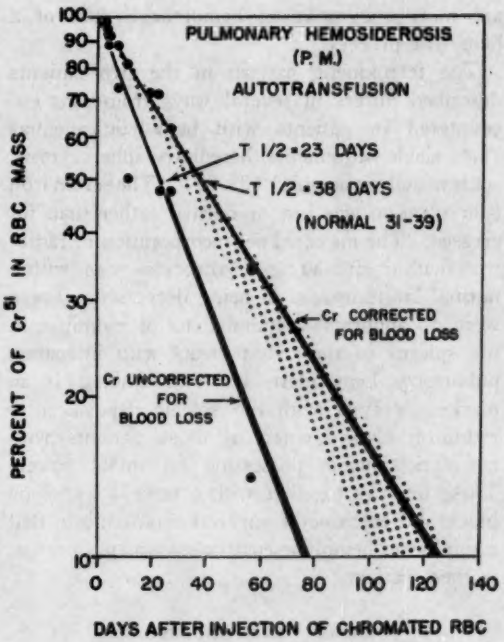

FIG. 11. Cr^{51} IN CIRCULATING RED BLOOD CELLS

DISCUSSION

The detection of relatively large amounts of radioactivity over the lung fields four months after the injection of radioiron at a time when the blood radioactivity was small indicates that erythrocyte iron was immobilized in the lung parenchyma. This finding is in agreement with the results of the chemical analyses of lungs in fatal cases of the disease, where large amounts of hydrolyzable iron have been found in the lungs (2). It has been demonstrated by histo-chemical techniques that the iron is mainly deposited as hemosiderin in macrophages. The macrophages are found within the alveolar spaces and within the septal walls. That the iron in the lungs was not used for blood regeneration is also substantiated by the persistence of a hypochromic anemia which continued during the four months of measurement. The anemia was subsequently virtually corrected by oral iron therapy. In contrast to the availability of storage iron for hemoglobin production in cases of idiopathic hemochromatosis, much of the iron in the hemosiderin deposits of patients with idiopathic pulmonary hemosiderosis is not available for hemoglobin regeneration.

Pulmonary deposition of erythrocyte iron conceivably may be the result of either hemorrhage or sequestration of erythrocytes in the lung. The abrupt onset and complete cessation of pulmonary erythrocyte iron deposition and the relative unavailability of the iron for hemoglobin formation

are more suggestive of hemorrhage than of a hemolytic process.

The ferrokinetic pattern in the two patients described differs in several ways from that encountered in patients with hemolytic anemias (*e.g.*, sickle cell anemia, hereditary spherocytosis, acute hemolytic anemia) (31, 37). The serum iron concentration was low or normal rather than increased. The maximal net incorporation of radioiron within circulating erythrocytes was within normal limits instead of being decreased. There were no abnormal accumulations of radioiron in the spleens of the two patients with idiopathic pulmonary hemosiderosis. This finding is in marked contrast with the splenic deposition of radioiron always noted in those patients with hemolytic anemia possessing an intact spleen. These findings together with a normal hemolytic index and erythrocyte survival time indicate that a significant hemolytic component was not present in these patients.

SUMMARY AND CONCLUSIONS

Two patients with idiopathic pulmonary hemosiderosis were studied during periods of remission and exacerbation to determine the mechanism of the anemia. Erythropoiesis and the movement of iron within the body was studied with Fe^{59}. Serial counting rate measurements were made in samples of plasma and erythrocytes, and at sites over a representative volume of bone marrow, liver, spleen and lung. Erythrocyte survival time was determined by labelling with radioactive sodium chromate (Cr^{51}) and by the Ashby differential agglutination method.

During a period of remission the plasma iron turnover and metabolic pathway of radioiron were normal in that a) iron was removed from the plasma at a normal rate, b) radioiron accumulated solely in the bone marrow, and c) over ninety per cent of the tracer iron appeared in the circulating erythrocytes within ten days. The survival time of the patient's erythrocytes in a healthy recipient and the life span of a healthy donor's erythrocytes in the patient were normal as determined by the Ashby technique.

The hemolytic index in one patient was normal at a time when there was a mild hypochromic anemia but no recent pulmonary symptoms.

When the radioiron studies were performed during an acute attack the path of radioiron was normal but movement was abnormally rapid. The plasma iron turnover was approximately triple the normal value. A significant rise in the counting rate over the spleen, a consistent finding in hemolytic anemias, was not observed. The survival time of the patient's chromated erythrocytes was normal when corrected for blood loss as determined by progressive decrease in erythrocyte radioiron activity.

In one patient exacerbations recurred after erythrocytes were labelled with Fe^{59}. The relapse was associated with a marked rise in the counting rate measurements over the lung fields; simultaneously, a corresponding decline in the radioactivity of the blood was observed. Monitoring over the lungs revealed that the counting rates were still high after four months, while only ten per cent of the original tracer iron concentration remained in the circulating erythrocytes. Together with this redistribution of iron, a hypochromic anemia continued until it abated in response to the oral administration of iron.

The studies indicate that: a) during exacerbation, the excessive plasma iron turnover and iron kinetic pattern are characteristic of an iron deficiency anemia and hemorrhage, but not of hemolysis; b) during remission there is no disturbance in erythropoiesis or erythrocyte survival; c) the spleen had no manifest role in the mechanism of the anemia; d) iron which is lost from the circulating blood into the lung parenchyma is poorly re-utilized despite a continuing iron deficiency anemia.

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STUDIES ON THE IMMUNOCHEMICAL BASIS OF ALLERGIC REACTIONS TO DEXTRAN IN MAN [1, 2]

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Early in the use of certain purified dextran solutions as plasma volume expanders in the treatment of shock in man, it was observed that immediate type allergic reactions could be produced (1–4). The then available clinical dextrans of Swedish and British origin had been found to give a high incidence of allergic reactions, while a product of American manufacture produced a very low incidence (3, 4). The reason for this difference remained obscure.

Studies on the antigenicity of purified dextrans showed them to be non-antigenic in the rabbit (5, 6) and guinea pig (6), whereas initial injection in rats gave an unusual type of allergic reaction (7–9) characterized by rapid onset of edema of the paws and snout.

Studies by Kabat and Berg (10, 11) which were confirmed by Maurer (12) demonstrated that highly purified dextrans were antigenic in man and that injection of 1 mg. of native or clinical dextran gave rise to precipitating antibodies and resulted in the development of wheal and erythema type skin sensitivity. It was further shown that a significant proportion of individuals showed small amounts of precipitating antibody to dextran (cf. 13) and wheal and erythema type sensitivity before any known deliberate contact with dextran.

In view of the interest in dextran solution as a plasma volume expander in the therapy of shock, and since the basis of the systemic reactions to dextran was not understood, and methods of commercial manufacture of dextran solution frequently failed to yield products which did not produce allergic reactions, it became important to evaluate the possible role of existing skin sensitivity and circulating antibody to dextran in relation to systemic reactions to infusions of dextran in a group of normal individuals. A cooperative study sponsored by the Subcommittee on Shock of the National Research Council and the Armed Services was undertaken.[1]

Earlier findings (3, 4) were confirmed that the incidence of generalized allergic reactions varied quite sharply with the type of dextran and manufacturing process used at that time. With a dextran giving a high incidence of systemic allergic reactions on intravenous infusion, a high statistical correlation was found between allergic reactions and initial skin sensitivity to certain native dextrans and also between allergic reactions and initial circulating precipitin levels as determined with certain dextrans. Clinical dextrans gave a low incidence of positive skin test reactions, whereas native dextrans gave a high incidence of positive reactions. Hehre and Neill (cited in Reference 2) had noted some positive skin reactions to native dextran in man.

A correlation between the incidence of skin sensitivity and the structure of the native dextran was also found. From an interpretation of the

[1] The data in this paper were presented in reports to the National Research Council Subcommittee on Shock by Drs. E. A. Kabat, G. M. Turino and A. B. Tarrow in January and April, 1952, and by Dr. P. H. Maurer in an interim report to the Chief, Research and Development Division, Office of the Surgeon General, U. S. Army, dated June 1, 1954.

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findings, it became possible to define the basis of specifications for clinical dextran manufacture with respect to selection of *Leuconostoc mesenteroides* strain, and upper and lower limits of molecular size. Adherence to these specifications permitted the manufacture of dextran giving a negligible incidence of allergic reactions in clinical use.

METHODS

Materials

A. Clinical dextrans for infusion were:

1) Swedish dextran Pharmacia Co.
   Lots 5402Y
   Z 6932 A
   A 8745 C

2) Commercial Solvents Corporation dextrans.
   Lot No. 255F4—molecular weight by light scattering [4]
   Average, 59,000
   High, 10.6 per cent 175,000
   Low, 12.3 per cent 22,780
   Lot No. 258A7
   Average, 87,800
   High, 11.3 per cent 263,000
   Low, 8.9 per cent 25,700

3) NRRL B742 clinical
   Average, 61,000
   High, 7.8 per cent 100,000
   Low, 7.9 per cent 30,000

Swedish dextran was produced by a strain of *L. mesenteroides* which yielded a native dextran (3079^{r}) with 88 per cent 1→6-like, 5 per cent 1→4-like and 7 per cent 1→3-like linkages, giving a ratio of 1→6 to non-1→6 linkages of 7.5 (14), while the Commercial Solvents Corporation dextran was prepared from the NRRL B512 strain which yielded a dextran containing 96 per cent of 1→6 linkages and 4 per cent of 1→3-like linkages (15) to give a ratio of 24. Lot 258A7 was deliberately prepared to have higher average and upper fraction molecular weights than the usual clinical Commercial Solvents dextran.

Data supplied by the Pharmacia Company and by Dr. S. G. Weissberg of the National Bureau of Standards (cf. National Bureau of Standards Report 1160 [1951]) indicated that batch 150 had 8.1 per cent, and batch 156 had 4.0 per cent of material with an intrinsic viscosity of 0.395; batch 168 had 5 per cent of material with an intrinsic viscosity of 0.364. From the data of Ingelman and Halling (16), Dr. Michael Wales found an intrinsic viscosity of 0.390 to correspond to a sedimentation constant of 6.4 or a molecular weight of 210,000 for a comparable Swedish dextran batch 155. On fractionation of a similar batch of Swedish dextran Y5740A which was studied by sedimentation equilibrium it was estimated that about 0.3 per cent of the material had a molecular weight greater than 500,000. Thus the Swedish preparations in the study contained appreciable amounts of higher molecular weight material than did Commercial Solvents dextran lot 255F4.

B. Dextrans for skin tests: Saline solutions containing 1 mg. per ml. of native dextrans NRRL B742, B1255, B512 from Dr. Allene Jeanes, native Swedish dextran 3079, clinical Commercial Solvents dextran and those Swedish clinical dextrans used above were prepared with 0.25 per cent phenol as preservative. A solution of Leuconostoc protein from an ultrasonically disintegrated culture of *L. mesenteroides* strain D grown on sucrose-free media after three passages through dextrose broth, was kindly supplied by Dr. Joel Warren of the Army Medical Graduate School at Walter Reed Army Medical Center, and was prepared for skin testing in a similar manner, and used at a concentration of 10 μg. protein per ml. (1.6 μg. N per ml.). Phenolized saline was used as a control. The nitrogen contents of native dextrans NRRL B742, B1255, B512 and native Swedish dextran were 0.003, 0.025, 0.004 and 0.05 per cent, respectively; these data are tabulated in references 10 and 11. Clinical dextrans are required to have less than 0.016 per cent N to be acceptable for use in man.

Experimental subjects

Two hundred and seven Air Force inductees, 17 to 26 years of age, who had never received an infusion of dextran volunteered for the experiment. From 51 of these the routine course of immunization required on induction was withheld until after the completion of the study. In addition, 33 subjects who had received dextran infusions from 4 to 23 months previously in earlier studies (3, 4) volunteered to receive a second infusion. The combined results of the two studies carried out three months apart in January and April, 1952, are presented.

Design of experiment

Studies were carried out in a ward at the Lackland Air Force Base Hospital, San Antonio, Texas, except for a small number of the re-infusions which were carried out at the Walter Reed Army Medical Center, Washington, D. C. Groups of approximately 50 subjects were studied at one time. An initial blood sample of 200 ml. was taken sterilely. The serum from these bleedings was prepared, frozen and shipped to Dr. Paul H. Maurer at the University of Pittsburgh by air express. Upon receipt of the sera, phenol to 0.25 per cent and merthiolate to a concentration of 1:10,000 were added and the sera kept frozen until used. Each subject then received nine intracutaneous injections of the various native or clinical dextrans, Leuconostoc protein, or phenolized saline along both arms. The 101 subjects in the first study were not skin tested with Leuconostoc protein. Resulting wheal and erythema reactions were evaluated 15 to 20 minutes later and were graded from negative to 4+,

[4] Data supplied by Dr. S. G. Weissberg, National Bureau of Standards, and Dr. F. Senti, Northern Regional Research Laboratory of the Department of Agriculture.

based on measurements of the diameter of the wheal and erythema reaction. Doubtful reactions were graded ±. Following the reading of the skin tests, infusion of dextran by vein was started; 20 to 30 minutes were required for administration of 500 ml. of 6 per cent dextran. Individuals were observed closely for the appearance of signs and symptoms of allergic reactions. Symptoms and signs resembled those described in detail by Tarrow (4) and others (1–3) and included flushing, headache, urticaria and angioneurotic edema, rhinitis, lacrimation, tightness of chest, wheezing and pulmonary rales, hypotension and syncope on standing, swelling of extremities, nausea and vomiting. Systemic reactions were classified as mild, moderate or severe depending upon the number and severity of the signs and symptoms. A conspicous feature of the systemic reaction was that a flare-up of the positive skin test sites was frequently noted. When systemic reactions appeared rapidly and were of appreciable severity, infusion of dextran was discontinued and Benadryl® and/or ephedrine were administered parenterally, as indicated. Subjects remained under observation for about 24 hours. Delayed skin reactions were not studied and only occasional subjects reported symptoms 18 hours or longer after infusion. Tarrow (4) reported pain in joints, abdominal cramps and aching shoulders in a small proportion of cases up to 24 hours post infusion. One month after infusion a second 200-ml. blood sample was taken and processed as above. This procedure was also followed with the 33 individuals who received re-infusions of dextran. This group, however, was divided so that half of the 15 individuals who had received an earlier infusion of Swedish dextran received a second infusion of Swedish dextran and the other half received Commercial Solvents dextran; while all 18 individuals who had previously had Commercial Solvents dextran and one who had previ-

TABLE I

Relation of skin sensitivity to intracutaneous injection of various dextrans, and systemic allergic reactions on infusion of dextran

Dextran used for skin test	Skin test*	Systemic reactions		χ^2	P†
		Negative	Positive		
Experiment 1—Infusion Swedish Dextran					
Native Swedish 3079	−	45	26	16	<0.00004
	+	5	25		
NRRL B1255	−	41	21		
	+	9	30		
NRRL B742	−	24	4	18	<0.00004
	+	26	47		
NRRL B512 (Native Commercial Solvents)	−	48	44	2	>0.05
	+	2	7		
Clinical Swedish	−	49	48		
	+	1	3		
Clinical Commercial Solvents	−	49	48		
	+	1	3		
Experiment 2—Infusion Commercial Solvents Dextran					
Native Swedish 3079	−	40	2	4.8	0.025
	+	51	14		
NRRL B1255	−	20	2		
	+	71	14		
NRRL B742	−	10	0		
	+	81	16		
NRRL B512 (Native Commercial Solvents)	−	63	7	2.8	0.1
	+	28	9		
Clinical Swedish	−	82	7	17.6	0.00017
	+	9	9		
Leuconostoc protein	−	10	1		
	+	81	15		

* ± reactions were tabulated as negative.
† P = 0.002 for χ^2 = 10.0 with one degree of freedom.

ously received British dextran, received a second infusion of Commercial Solvents dextran.

Quantitative precipitin tests were carried out by the Heidelberger and MacPherson (17) micromodification of the quantitative precipitin test, using quantities of the various native or clinical dextrans (10–12). Although 3.0 ml. of serum was used for each analysis the results are expressed in micrograms of antibody nitrogen per ml. of serum.

RESULTS

Results as presented are a summary of the findings. Complete observations on each individual and the antibody analyses appear in the reports to the National Research Council.[1]

Of the 101 individuals who received an infusion of Swedish dextran, 32 showed mild reactions, 15 moderate and 4 showed severe reactions. No difference in incidence of reactions related to the routine Armed Forces immunizations was found. Of 100 individuals who received Commercial Solvents dextran, 12 showed mild reactions and 2 showed severe reactions. Many of the mild reactions consisted only of one or two hives. The two severe reactions with Commercial Solvents dextran occurred in the group of 30 individuals who had received Commercial Solvents dextran, lot 258A7 with the higher molecular weight range. Of the 7 individuals who received clinical NRRL B742 dextran, one showed a mild and one a moderate reaction.

Table I shows the incidence of skin sensitivity to the various dextrans used for skin tests in individuals who showed positive and negative systemic reactions.

Figure 1 summarizes the distribution among the systemic reactors and non-reactors with respect to the severity of the skin test. The degree of the systemic reactions is also indicated. It is clearly evident from Table I that there is a high degree of correlation between systemic allergic reactions to infusion of Swedish dextran and previously existing skin sensitivity to native Swedish dextran and to native dextrans NRRL B1255 and B742. Native NRRL B512 and the two clinical dextrans gave much lower incidences of initial skin sensitivity and no correlation with systemic reactions was found.

In the group of individuals who received infusions of Commercial Solvents dextran, a high correlation with pre-existing skin sensitivity to clinical Swedish dextran and a moderate correla-

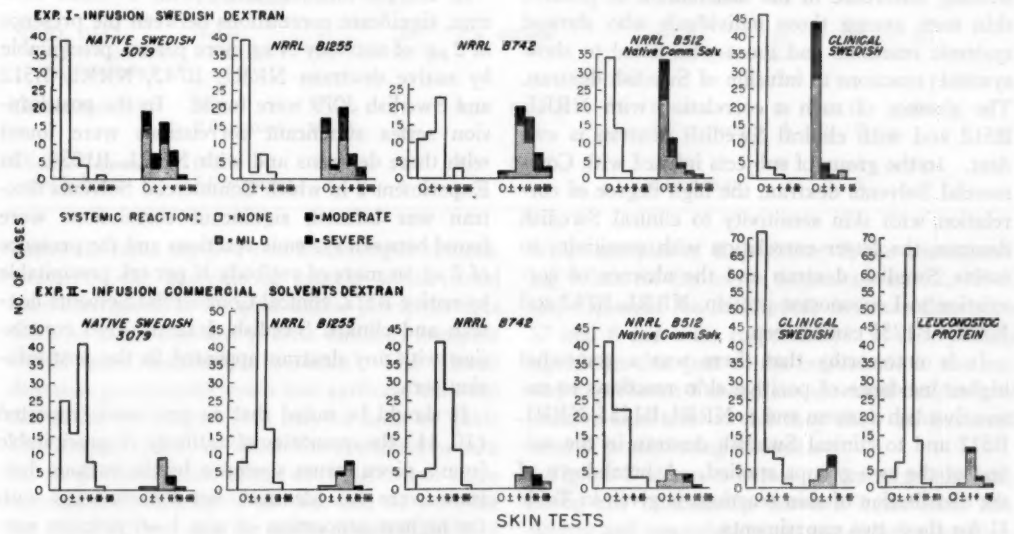

FIG. 1. DISTRIBUTION OF SKIN TESTS TO VARIOUS DEXTRANS IN RELATION TO SYSTEMIC REACTIONS ON SUBSEQUENT INFUSION OF DEXTRAN

For each dextran the unshaded areas represent the non-reactors and the shaded areas the degree of system reaction as indicated.

TABLE II

Distribution of pre-existing skin reactions in individuals studied in experiments 1 and 2

Dextran	Experiment number	Degree of skin sensitivity (Number of individuals)					
		−	±	+	++	+++	++++
Native Swedish 3079	1	58	13	20	9	1	
	2	25	17	52	11	2	
NRRL B1255	1	56	6	27	9	3	
	2	9	13	60	23	2	
NRRL B742	1	10	15	42	17	10	3
	2	3	7	43	31	13	3
NRRL B512	1	67	25	6	2	1	
	2	42	28	25	9	3	
Clinical Swedish	1	90	7	2	2		
	2	74	15	17	1		
Leuconostoc protein	2	3	8	78	10	2	

tion to pre-existing skin sensitivity to native Swedish dextran was found. No significant correlation could be demonstrated with Leuconostoc protein or the other dextrans. The high degree of significance with respect to skin sensitivity and allergic reactions is further illustrated in Figure 1. It can be seen that with native Swedish dextran 3079, NRRL B1255 and NRRL B742, there is a striking difference in the distribution of positive skin tests among those individuals who showed systemic reactions and those who failed to show systemic reactions to infusion of Swedish dextran. The absence of such a correlation with NRRL B512 and with clinical Swedish dextran is evident. In the group of subjects infused with Commercial Solvents dextran, the high degree of correlation with skin sensitivity to clinical Swedish dextran, the lesser correlation with sensitivity to native Swedish dextran and the absence of correlation to Leuconostoc protein, NRRL B742 and NRRL B1255 can be seen.

It is noteworthy that there was a somewhat higher incidence of positive skin reactions to native Swedish dextran and to NRRL B1255, NRRL B512 and to clinical Swedish dextran in the second of the two groups studied. A breakdown of the distribution of skin reactions is given in Table II for these two experiments.

It is also of interest that the incidence of positive skin tests of individuals before infusion of dextran was higher with those native dextrans having a higher proportion of non 1→6 linkages. The proportion of positive skin tests was unrelated to the nitrogen content of the native dextrans, B742 with the lowest nitrogen giving the highest incidence of positive skin reactions.

Table III summarizes data on the relation between systemic allergic reactions and circulating precipitins to various dextrans before infusion and one month after infusion of dextran.

In the pre-infusion series with Swedish dextran, significant correlations between the presence of 2 μg. of antibody N or more per ml. precipitable by native dextrans NRRL B742, NRRL B512 and Swedish 3079 were found. In the post-infusion series significant correlations were found with these dextrans and with NRRL B1254. In Experiment 2 in which Commercial Solvents dextran was infused, significant correlations were found between systemic reactions and the presence of 2 μg. or more of antibody N per ml. precipitable by native B512, clinical Commercial Solvents dextran and clinical Swedish dextran. No correlation with any dextran appeared in the post-infusion sera.

It should be noted that as previously reported (10, 11) the quantities of antibody N precipitable from a given serum specimen by the various dextrans were not identical, but the dextrans with the highest proportion of non 1→6 linkages precipitated considerably more antibody than did those with fewer non 1→6 linkages. Native dextran NRRL B742 with the lowest nitrogen content precipitated the most antibody of the various

TABLE III

Relation of circulating precipitins to various dextrans before and one month after infusion of dextran and allergic reactions encountered on infusion

Dextran used for precipitin test	Pre-infusion precipitin level	Systemic reaction		χ^2	P	Precipitin levels one month post-infusion	Systemic reaction		χ^2	P
		Negative	Positive				Negative	Positive		
	μg. Ab. N/ml.					μg. Ab. N/ml.				
					Experiment 1—Infusion Swedish Dextran					
Native Swedish 3079	0–1.9	39	31	4.0	0.046	0–1.9	29	22	5.1	0.024
	2.0 or more	6	15			2.0 or more	4	13		
NRRL B1254	0–1.9	41	43			0–1.9	30	24	6.2	0.013
	2.0 or more	3	1			2.0 or more	2	10		
NRRL B742	0–1.9	39	14	26.6	0.00004	0–1.9	20	9	8.8	0.0038
	2.0 or more	7	32			2.0 or more	13	25		
NRRL B512 (Native Commercial Solvents)	0–1.9	44	35	5.8	0.016	0–1.9	31	20	11.3	<0.002
	2.0 or more	2	11			2.0 or more	2	15		
Clinical Swedish	0–1.9	44	44			0–1.4	33	31	1.9	0.16
	2.0 or more	2	2			2.0 or more	1	4		
Clinical Commercial Solvents	0–1.9	46	45			0–1.9	35	31	3.1	0.07
	2.0 or more	0	1			2.0 or more	0	3		
					Experiment 2—Infusion Commercial Solvents Dextran					
Native Swedish 3079	0–1.9	60	8	2.6	0.11	0–1.9	63	10	0.39	0.53
	2.0 or more	28	9			2.0 or more	26	5		
NRRL B1254	0–1.9	54	9	<0.3		0–1.9	75	12	0	
	2.0 or more	32	8			2.0 or more	13	2		
NRRL B742	0–1.9	27	2	3.1	0.08	0–1.9	30	5	0	
	2.0 or more	59	16			2.0 or more	59	9		
NRRL B512 (Native Commercial Solvents)	0–1.9	57	6	7.3	<0.009	0–1.9	67	12	0.4	
	2.0 or more	31	12			2.0 or more	22	3		
Clinical Swedish	0–1.9	75	12	4.3	0.04	0–1.9	85	14	0	
	2.0 or more	12	6			2.0 or more	4	0		
Clinical Commercial Solvents	0–1.9	86	14	11.4	0.002	0–1.9	87	14	0	
	2.0 or more	2	4			2.0 or more	2	1		

native dextrans used. From the nitrogen content, moreover, the amounts of protein which could have been added in the quantitative precipitin tests were so small that any protein-antibody complex would have remained in solution. The clinical dextrans precipitated much less antibody than did the native dextrans. This may be seen in Table III, Experiment I, in which for pre-infusion sera the proportion of individuals showing two or more micrograms antibody N per ml. was 42 per cent for B742, 23 per cent for native Swedish 3079, 5 per cent for native B1254 and 14 per cent for native B512, while for clinical Swedish and clinical Commercial Solvents dextrans the percentages were 4 and 1, respectively. In the pre-infusion serum samples of Experiment 2, the corresponding values for the native dextrans were 72, 35, 39 and 41, while for the clinical dextrans they were 17 and 6 per cent, respectively. It is especially significant that just as the individuals in the second experiment showed an increased incidence of positive skin tests so did they show an increased level of precipitating antibodies.

Figure 2 shows a series of scattergrams indicating the relationship between initial skin sensitivity and pre-infusion or post-infusion antidextran levels to B742 with the systemic allergic reactions experienced. It is evident that individuals who showed a high degree of skin sensitivity or a high level of pre-existing precipitating antibody

tended to have positive systemic allergic reactions. The striking difference in the incidence of allergic reactions on infusion of Swedish dextran as compared with Commercial Solvents dextran is evident. Moreover, inspection of the upper and lower scattergrams with Swedish dextran shows a substantial shift toward the appearance of higher levels of circulating precipitins one month after infusion. On the other hand, the similar comparison of the upper and lower scattergrams obtained with Commercial Solvents dextran shows a trend toward the existence of lower antidextran levels to B742 one month after infusion. Thus it would appear that infusion of Swedish clinical dextran provoked an anamnestic rise in antidextran level one month following infusion, while similar infusion of Commercial Solvents dextran generally had no such effect. Four individuals in the latter group showed an anamnestic rise of over 2 μg. of antibody N per ml. as compared with fifteen individuals in the former group. Twenty individuals in the second experiment showed a decline of 2 μg. antibody N per ml. or greater, as compared with seven individuals in the first experiment. Scattergrams similar to those in Figure 2 for native Swedish dextran and native Commercial Solvents dextran were obtained.

A group of 33 individuals who had received infusions either of Swedish dextran or of Commercial Solvents dextran 4 to 23 months previously in the studies of Pulaski, Baker, Tarrow, and Amspacher (3, 4) volunteered to undergo skin testing and a second infusion of dextran. Table IV shows the distribution of skin sensitivity to the various dextrans. Comparison of the results in Table II for Experiment 2 done at the same time shows no essential difference in the incidence of skin sensitivity. Quantitative precipitin studies on serum taken prior to the second infusion showed no significant differences in antibody level as compared to the groups which had not been previously infused. Table V shows that prior

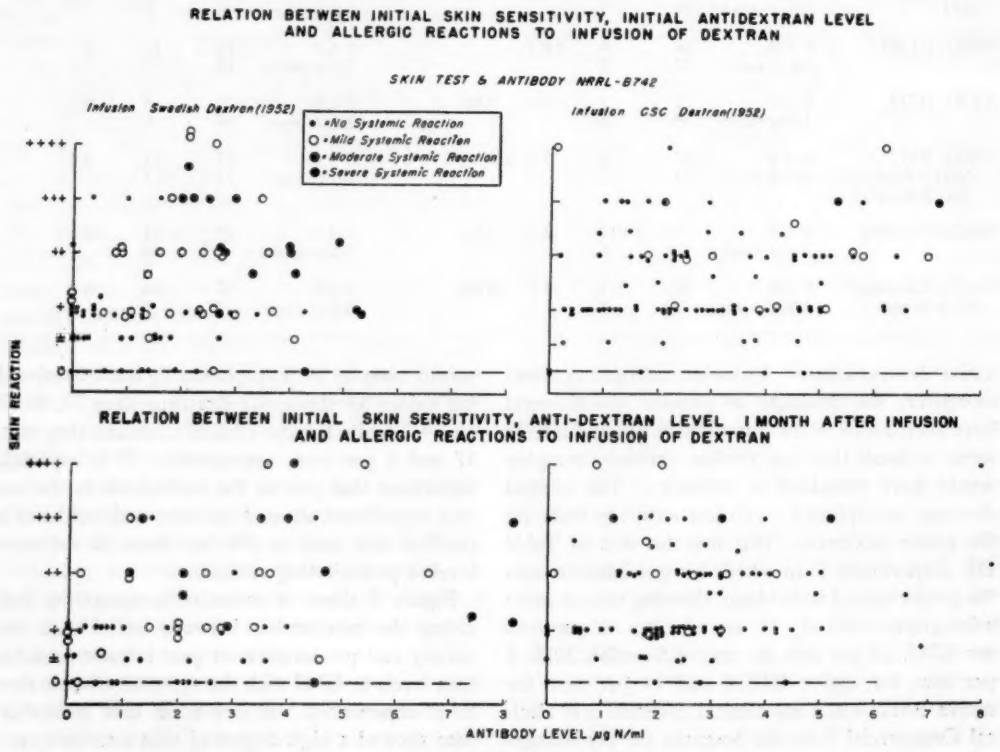
RELATION BETWEEN INITIAL SKIN SENSITIVITY, INITIAL ANTIDEXTRAN LEVEL AND ALLERGIC REACTIONS TO INFUSION OF DEXTRAN
SKIN TEST & ANTIBODY NRRL-B742
Infusion Swedish Dextran(1952)
Infusion CSC Dextran(1952)
• No Systemic Reaction
○ • Mild Systemic Reaction
● • Moderate Systemic Reaction
● • Severe Systemic Reaction
SKIN REACTION
RELATION BETWEEN INITIAL SKIN SENSITIVITY, ANTI-DEXTRAN LEVEL 1 MONTH AFTER INFUSION AND ALLERGIC REACTIONS TO INFUSION OF DEXTRAN
ANTIBODY LEVEL μg N/ml

FIGURE 2

TABLE IV

Distribution of skin tests prior to re-infusion in 33 individuals previously infused with dextran

Dextran	Degree of skin sensitivity (Number of individuals)					
	−	±	+	++	+++	++++
Native Swedish 3079	14	0	18	1		
NRRL B1255	9	6	13	5		
NRRL B742	7	3	13	8	1	1
NRRL B512	23	2	7	1		
Clinical Swedish	25	4	4			
Leuconostoc protein	0	0	25	8		

infusion of either Swedish or Commercial Solvents dextran within the time interval studied did not result in any enhanced susceptibility to allergic reactions on re-infusion of dextran. Three of the four positive reactions which resulted on re-infusion of Swedish dextran were of the same degree of severity as had previously been noted from the first infusion of Swedish dextran. The fourth individual showed only one hive from the second infusion.

DISCUSSION

The results outlined above clearly established that it is possible to produce a dextran suitable for clinical use which gives a low incidence of allergic reactions in a population, a large proportion of which showed initial skin sensitivity of the wheal and erythema type and whose sera contained precipitating antibodies to dextran. This preparation from a strain of Leuconostoc (NRRL B512) yielded a dextran having the lowest incidence of non-1→6 linkages (15) and hence was the most linear dextran available. Furthermore, in the random population native dextran from this strain showed the lowest incidence of wheal and erythema skin reactions of any of the native dextrans tested. With respect to circulating precipitins, however, the number of individuals showing two or more micrograms of antibody N per ml. was about the same for native Swedish, native B512 and native B1254 dextrans and these were considerably fewer than the number of individuals showing 2 or more micrograms of antibody N per ml. with B742. Partial hydrolysis of Swedish or Commercial Solvents dextrans to produce a dextran

TABLE V

Incidence of adverse systemic reactions to a second infusion of dextran

Initial dextran infusion	Interval following first infusion	Dextran re-infused	Number of individuals	Reactions			Incidence of reactions
				Mild	Moderate	Severe	
	(*months*)						
Commercial Solvents	4½	Commercial Solvents	10*,†	0	0	0	0/10
Commercial Solvents	9½ to 23	Commercial Solvents	7	0	0	0	0/7
Swedish	11 to 21	Commercial Solvents	7‡,§	0	0	0	0/7
English	17	Commercial Solvents	1	0	0	0	0/1
Swedish	11 to 21	Swedish	8‖	2	1	1	4/8

* One individual had received two previous infusions of Commercial Solvents dextran, one 11 months and the other 4½ months earlier.

† One individual had had a severe reaction to the initial infusion of Commercial Solvents dextran.

‡ One individual had received two previous infusions of dextran, one of Swedish dextran 12 months and the other of Commercial Solvents dextran 11 months earlier.

§ Two of these individuals had had a severe and a mild reaction on initial infusion of Swedish dextran 13½ months earlier.

‖ Three of these individuals had had severe, mild and moderate reactions to an initial infusion of Swedish dextran 18, 13, and 5 months earlier. The same three individuals experienced similar reactions to re-infusion of dextran.

suitable for use as a plasma volume expander produced a dramatic reduction in the capacity of the material to induce wheal and erythema reactions or to precipitate the circulating antidextran in the same population (Tables II and III). It is noteworthy that the dextran which produced the lowest incidence of allergic reactions (CSC 255F4) had been sharply fractionated to remove materials of high molecular weight, while the dextran which gave the high incidence of allergic reactions (Swedish) had not been so fractionated and contained appreciable quantities of materials with very high molecular weights. The lot containing intermediate-sized material (CSC 258A7) gave two severe reactions in thirty individuals tested. In an independent study, Kabat and Bezer (18) have shown that a substantial decrease in antigenicity of B512 dextran occurs with molecular weights below 90,000, and Maurer (12) has reported definite antigenicity for dextran 258A7 obtaining levels of about 10 or more μg. antibody N per ml. in three of six individuals injected. It would then appear that choice of dextran producing microorganism, degree of hydrolysis, and extent of removal of high molecular weight constituents are important factors in the manufacture of reaction-free dextran.

The occurrence of precipitins to dextran and of initial skin sensitivity to dextran in the population raise the question as to the origin of these antibodies. Since dextran has been shown by Neill, Hehre, Sugg, and Jaffe (19, 20) to be a contaminant of commercial sugar, and since these workers have isolated dextran-producing organisms from the gastrointestinal tract (21, 22), ample opportunity exists for the development of an antibody response. Furthermore, since dextrans are known to cross-react with Types II, XII, XX, IX, XXII, etc. rabbit and horse antipneumococcal sera (19, 20, 23), the precipitable antibody and skin sensitivity to dextran in human subjects may theoretically in some instances be attributable to previous contact or infection with these organisms. On the other hand, Maurer (12) found that immunization of humans with dextran did not affect the Type II, XII or XX antipolysaccharide levels, nor did absorption with Type II, XII, or XX pneumococcal polysaccharides reduce the antidextran level. It is not surprising that a linear dextran should give a lower incidence of skin sensitivity than a more highly branched dextran, since the latter would possess a multiplicity of structures not present in the former and thus would increase the probability of its being able to react with antibodies produced to the heterogenous array of antigenic stimuli encountered at random in the human environment.

Although the data clearly indicate a high statistical correlation between initial skin sensitivity to native dextrans and systemic allergic reactions, there are a sufficient number of exceptions so that the single skin test of 10 to 20 micrograms of dextran was of little prognostic significance in evaluating the occurrence of allergic reaction in response to injection of 30 Gm. of dextran. This is not surprising since a) the nature of the antigen to which the initial skin sensitivity developed is not known and, in all probability, is not the same as that used for skin test and infusion; b) the ratio of the infused dose to the skin test dose is unusually high, of the order of three million; c) only a single skin test dose was used.

Similar considerations obviously apply to the individual cases in which precipitating antibody levels and allergic reactions did not run parallel, although again a high statistical correlation was observed between these levels and allergic reactions. Thus the capacity of the various dextrans to precipitate the multiplicity of antibodies resulting from the unknown antigenic stimuli and the necessarily limited capacity to establish optimum quantities of dextran for precipitating these antibodies in the quantitative precipitin studies would contribute substantially to such individual variations.

The finding of an anamnestic rise in antibody level one month after infusion of Swedish dextran in a considerable proportion of individuals while no such rise was observed following infusion of Commercial Solvents dextran is in accord with evidence suggesting that the latter is less antigenic in man, although MacLeod (24) has found that the pneumococcus polysaccharides are capable of eliciting an anamnestic response in the rabbit despite their inability to produce a primary antibody response.

While the limited re-infusion data indicate that dextran may be re-infused up to 23 months following an initial infusion without difficulty, it is obviously impossible to extrapolate to periods be-

yond this. The generally lower antigenicity of the current commercial preparations of dextran prepared from the B512 strain according to American specifications provide additional assurance of its probable safety for clinical use. It should be emphasized that the Swedish preparations herein described were in use in 1951–1952. The current preparations of Swedish dextran differ in two important respects from these older preparations in that they are prepared from the B512 strain and are more sharply fractionated to remove high molecular weight constituents.

SUMMARY

1. A detailed protocol for elucidating the relationship between skin sensitivity and precipitating antibody in the random population to a carbohydrate antigen and their relation to the occurrence of allergic reactions on intravenous administration is described.

2. Significant correlations were established between a) initial skin sensitivity to certain native dextrans and the occurrence of systemic allergic reactions on administration of Swedish dextran; b) the presence in human sera of antibodies specifically precipitable by certain dextrans and the occurrence of systemic allergic reactions to clinical Swedish dextran; c) the structural complexity of native dextrans and the incidence of positive skin sensitivity in the random population.

3. Partial hydrolysis of native dextrans to the molecular weight range suitable for clinical use results in a striking diminution in their capacity to produce a positive wheal and erythema reaction in man.

4. In a population of comparable skin sensitivity and antibody levels a preparation of clinical dextran (Commercial Solvents) was encountered which gave a very low incidence of systemic allergic reactions. This appeared to be correlated with the selection of a strain of *Leuconostoc mesenteroides* (NRRL B512) producing a dextran with an unusually low proportion of non 1→6 linkages and with the elimination of materials of higher molecular weight range by fractional precipitation to give a product with a narrower molecular weight distribution.

5. In a small series of individuals receiving reinfusion of both Swedish and Commercial Solvents dextran no evidence of increased sensitization was found during an interval of up to 23 months between the two infusions.

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EFFECTS OF EIGHT-HOUR INTRAVENOUS INFUSIONS OF ACTH AND THE ADRENOCORTICAL STEROIDS IN NORMAL MAN. I. BASAL GASTRIC SECRETION, AND PLASMA AND URINARY PEPSINOGEN [1, 2]

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While there appears to be general agreement that adrenocortical *deficiency* results in a reduction of gastric secretory activity both in man (1–6) and in animals (7–11), no such unanimity of opinion exists regarding the effects of *increased* activity of the adrenal cortex on gastric secretion. Whereas some studies have shown that the administration of ACTH or of adrenocortical steroids may increase gastric acid and pepsin secretion (12–14), others have failed to show any significant increase in acid or pepsin output by the stomach during the administration of ACTH (15–17) or cortisone or hydrocortisone (18, 19). Part of the confusion regarding any stimulant effect of increased adrenocortical activity stems from the use of urinary pepsinogen (uropepsin) levels as an index of gastric secretion.

In this study the effects of 8-hour infusions of ACTH, hydrocortisone, corticosterone, prednisolone, and aldosterone in normal males on the volume, acid, pepsin, and viscosity of gastric juice were studied, together with simultaneous measurements of blood and urinary pepsinogen. These results were compared with the effects of infusion of the vehicle alone and contrasted to the effects of a 4-hour infusion of histamine. The results indicate that in general the hormones did not produce stimulation of gastric secretion compared with a known stimulant such as histamine.

An analysis of changes in urinary pepsinogen excretion indicates that the glucocorticoids increase the excretion of this substance. Furthermore, urinary pepsinogen has been found to be a poor index of gastric secretory activity for reasons which are discussed below.

METHODS

Sixteen normal adult male students were studied in the fasting state. In 15 of these subjects, each experiment was started between 7 and 8 A.M.; in the other, the experiments started at 11 A.M. To serve as a control period, the vehicle alone (1 per cent ethyl alcohol, 2.5 per cent dextrose, and 0.45 per cent NaCl in water) was infused for 3 hours, followed for 8 hours by the infusion of 25 international units of corticotropin (ACTH), 100 mg. hydrocortisone (F), 100 mg. corticosterone (B), 25 mg. prednisolone (Δ-F), or 400 μg. aldosterone in 1,000 ml. of the vehicle, followed in turn by a further 2 hours of infusion of vehicle. Thirteen subjects received ACTH; 11, hydrocortisone; 9, corticosterone; and 13, the vehicle alone. Eight subjects each had all four experiments, 2 of these also receiving prednisolone, and one of the latter an infusion of aldosterone as well. In further experiments on 6 of these subjects and 4 others, 1.2 mg. histamine base (as the diphosphate) in 500 ml. 0.9 per cent saline was infused over 4 hours after a one-hour control period.

Gastric tubes were passed either orally or nasally according to the subject's preference, and the gastric end positioned under fluoroscopic control in the proximal part of the pyloric antrum at the start of the experiment. At the end of each experiment the tube was again checked for position, and in no instance had it shifted significantly. Continuous aspiration of the stomach was maintained through the experiment and supplemented at half-hour intervals by hand suction with a syringe.

The consecutive half-hour samples of gastric juice were analyzed for free H^+ ion by electrometric titration to pH 3.3 against 0.1 N NaOH; the samples were then refrigerated and at the end of each experiment they were centrifuged for 15 minutes at 2,500 rpm and the viscosity of the supernate measured immediately at room temperature by measuring the time taken for a column of gastric juice to fall between two fixed points in a straight capillary tube (using 0.2 ml. fluid) (13). To determine the approximate changes in mucin concentration

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which might be responsible for the observed variations in viscosity of gastric juice by this method, the viscosity of a series of dilutions of hog mucin (Wilson 1701-W) in 0.04 N HCl was measured (Figure 1). In order to establish the validity of measuring the viscosity of the gastric juice by this method, the results of 127 samples from some of these experiments were compared with values obtained with an Ostwald viscometer (using 3.5 ml. fluid) at 37° C. in the same samples. The results, in terms of water as unity, were strictly comparable and a correlation coefficient gave an 'r' value of + 0.88. The standard error of 25 replicate measurements of one sample was ± 0.14 per cent of the mean. The viscosity readings were not influenced by centrifugation at 2,500 rpm for periods ranging from 10 to 50 minutes after the initial centrifugation, suggesting that the substances imparting the extra viscosity were in solution. Bile contamination in the amounts observed had no appreciable effect on the viscosity of gastric juice. It should be added that the method employed here is not recommended for comparing different fluids, but appeared to be adequate for the serial determination of the viscosity of gastric juice.

Icteric index and pepsin concentration of the gastric juice were measured the next morning, the former by the method described for plasma (20), the latter using 2 per cent hemoglobin substrate and a 1:50 dilution of gastric juice (21). The total eosinophil count, employing a phloxine stain, and the plasma pepsinogen (21) content of blood samples collected every 2 hours were determined. Samples of urine collected overnight before each and after most experiments and every 2 hours during each experiment were assayed for pepsinogen by the hemoglobin method (22), modified only by extending the incubation time to 30 minutes. Urinary 17-hydroxycorticoids (23) were measured in the ACTH experiments only.

Contamination by saliva was reduced by having the subjects expectorate at frequent intervals. The icteric index of the gastric juice was used as some measure of the degree of duodenal regurgitation, in preference to continuous drainage of the duodenum by a second tube, which would require frequent fluoroscopic control and which of itself may stimulate gastric secretion (24). When the acid and pepsin concentrations, respectively, were plotted against the icteric index it was found that normal duodenal regurgitation had no consistent effect on the concentration of either acid or pepsin. The icteric index was under 8.0 in 96 per cent of samples and under 3.0 in 80 per cent of samples in all the experiments. With this reassurance of the minimal role of duodenal regurgitation in neutralizing or diluting the gastric juice, all the samples were included in the calculations regardless of their bile contamination.

Calculations and statistical methods. In calculating the results, both the outputs and the mean concentrations of acid and pepsin for each hourly period were derived from the consecutive half-hour samples. The mean values include all the hourly samples in each 2 or 3-hour period in all the subjects. Thus, for 13 experiments 39 samples were used to calculate the mean and the standard deviation (S.D.) in the 3-hour control period and 26 samples in each successive 2-hour period. Because of the small numbers of figures in the prednisolone and aldosterone experiments these means have not been subjected to statistical testing.

To determine the variability in consecutive hourly collections of gastric juice volume, acid and pepsin output in the 13 *vehicle* (control) experiments, the coefficient of variation for each parameter in each subject was calculated. The mean (± S.D.) of the coefficients of variation for volume was 34 per cent ± 9 per cent; for pepsin output 35 per cent ± 6 per cent; and for HCl output 67 per cent ± 31 per cent. The greater variability in HCl output was due to the relatively large fluctuations in those subjects with the lowest output (under 1 mEq. per hr.); the higher the output, the steadier was the rate of secretion.

RESULTS

Eosinophil and 17-hydroxycorticoid responses (Table I)

All the subjects given ACTH had an adequate adrenocortical response as judged from the rise

TABLE I

Effect of intravenous infusions (a) on circulating eosinophils and (b) of ACTH on urinary excretion of 17-hydroxycorticoids

Substance infused	Control period		During infusion				After infusion		
	−3 hr.	0 hr.	0–2 hr.	−4 hr.	−6 hr.	−8 hr.	+2 hr.	+3 hr.	+12 hr.
	(a) Mean total eosinophil count (*cells/cu. mm.*±*S.D.*)								
Vehicle	193±100	152±76	150±69	140±64	171±82	155±82	120±70		
ACTH	132± 62	115±49	99±31	55±30	23±21	12± 7	5± 6		
Corticosterone	167± 49	149±50	147±53	143±79	116±74	123±78	124±54		
Hydrocortisone	175± 54	172±70	142±78	50±45	21±33	12±13	16±11		
Prednisolone	260	225	230	151	97	47			
Aldosterone	260	235	227	286	297	269	168		
	(b) Mean urinary excretion of 17-hydroxycorticoid (*mg./hr.*±*S.D.*)								
ACTH (Overnight .015±.03)	.058± .07	.082±.11	.333±.23	.763±.53	.961±.61	1.633±.90	1.501±1.0	.865±.66	.601±.42

TABLE II

Effect of intravenous infusions on volume of gastric juice

Substance infused	No. of studies	Mean volume of gastric juice (*ml./hr.*±*S.D.*) Control period 3 hr.	During infusion 0–2 hr.	−4 hr.	−6 hr.	−8 hr.	After infusion +2 hr.
Vehicle	13	63±36	59±24	67±30	54±27	65±24	71±26
ACTH	13	53±34	51±41	53±33	55±33	74±37*	78±38†
Corticosterone	9	44±21	53±19*	49±18	54±21*	52±16*	65±29†
Hydrocortisone	11	54±46	53±29	57±42	53±29	58±35	63±40
Prednisolone	2	60±29 (5 hr.)	47±10	37± 1	48±18	78± 4	
Aldosterone	1	63± 8 (4 hr.)	29± 3 (1 hr.)	30± 3 (2 hr.)	34± 6 (3 hr.)	56± 4 (4 hr.)	85± 2
Histamine I.V.	10	66±38 (1hr.)	166±51†	225±72†	201±55†	202±94†	

* $p < 0.05$ } Significance of increase over control period by Student's 't' test. All other differences from control periods are not significant.
† $p < 0.01$ }

in 17-hydroxycorticoids and the fall in circulating eosinophils. A similar eosinophil response was elicited by the administration of hydrocortisone and prednisolone. No significant eosinophil changes were noted with the vehicle infusions or following the administration of corticosterone or aldosterone.

Volume of gastric juice

Table II details the mean hourly volume of gastric juice aspirated in the 3-hour control period and in each succeeding 2-hour period of the experiments. The volumes were significantly increased over the control period in the last 2 hours of ACTH infusion and in the succeeding 2 hours, as well as during the entire period (except the second 2 hours) of the corticosterone infusion and in the 2 hours following its infusion. The magnitude of these increases, though statistically significant, did not approach 25 per cent of the increases noted with histamine. There were no significant increases in the volume of gastric secretion in either the vehicle or in the hydrocortisone or the prednisolone experiments.

Output of hydrochloric acid (Table III)

The output of acid did not change significantly from the corresponding control values during the infusion of any of the hormones. The only statistically significant rise in the output of acid occurred in the 2 hours after the end of the corticosterone infusion. Even in this period, however, the actual output of acid was of the same order of magnitude as in the corresponding period of the

TABLE III

Effect of intravenous infusions on gastric output of hydrochloric acid

Substance infused	No. of studies	Mean gastric output of HCl (*mEq./hr.*±*S.D.*) Control period 3 hr.	During infusion 0–2 hr.	−4 hr.	−6 hr.	−8 hr.	After infusion +2 hr.
Vehicle	13	2.38±2.6	1.39±1.3	2.18±2.12	1.74±1.78	2.14±1.73	2.56±1.29
ACTH	13	1.94±2.3	1.67±2.9	1.55±1.97	1.82±2.07	2.52±2.52	3.14±3.06
Corticosterone	9	1.67±1.59	1.60±1.1	2.27±1.8	2.04±1.3	2.16±1.2	2.87±1.8†
Hydrocortisone	11	2.39±3.2	2.11±2.19	2.56±3.3	2.17±2.8	2.35±2.6	2.48±2.5
Prednisolone	2	1.35±.67 (5 hr.)	1.05±.33	1.0 ±.41	1.72±.97	3.02±.54*	
Aldosterone	1	1.42±.59 (4 hr.)	1.07±.15 (1 hr.)	1.23±.10 (2 hr.)	1.91±.43 (3 hr.)	2.87±.40 (4 hr.)	3.53±.45†
Histamine	10	1.0 ±.62 (1 hr.)	15.9±6.3†	24.8±8.0†	24.4±10.6†	21.0±12.0†	

* $p < 0.05$ } Significance of increase over control period by Student's 't' test. Other differences not significant.
† $p < 0.01$ }

TABLE IV

Effect of intravenous infusions on output of gastric pepsin

Substance infused	No. of studies	Mean output of gastric pepsin (*P.U. (thousands) per hour* ±*S.D.*)					
		Control period	During infusion				After infusion
		3 hr.	0–2 hr.	–4 hr.	–6 hr.	–8 hr.	+2 hr.
Vehicle	13	77.7±53.9	74.5±39	95.8±59	88.5±42	97.1±43	108±36.6
ACTH	13	52.7±27.8	51.9±29	56.3±31.8	62.0±27.6	66.6±32.4	75.7±34.9†
Corticosterone	9	62.4±24.1	73.0±25.8	80.4±26.3*	87.8+35.1†	88.4±38†	102.1±41.6†
Hydrocortisone	11	65.1±37.4	70.6±35.6	69.4±41.3	63.6±33.7	61.9±45.6	67.2±51
Prednisolone	2	65±38.9 (5 hr.)	53.5±18.6	58.3±24.8	75±41.4	89.5±33.6	
Aldosterone	1	59.5±21.6 (4 hr.)	67±1 (1 hr.)	66±3 (2 hr.)	77±14 (3 hr.)	114±6.4 (4 hr.)	111±11
Histamine	10	61.8±31.5 (1 hr.)	214±76†	244±89†	218±78†	257±107†	

* $p < 0.05$ } Significance of increase over control period by Student's 't' test. All other differences from mean control not statistically significant.
† $p < 0.01$ }

vehicle experiments. Furthermore, none of the mean values of acid output approached 20 per cent of the sustained levels during the histamine infusion.

Output of pepsin (Table IV)

There was a small, progressive increase in the output of pepsin in all of the experiments, except those in which hydrocortisone was infused. When compared with the values during the corresponding control periods, the pepsin output was significantly increased during the last 6 hours of the corticosterone infusion and after the end of the infusions of corticosterone and of ACTH. The actual output of pepsin in the hormone experiments, however, was always lower than in the corresponding periods in the vehicle experiments, and the absolute increases observed in the hormone experiments were never greater than 25 per cent of the increases which occurred with the histamine infusions.

The composition of gastric juice

The over-all secretory activity of the stomach as judged by the *output* of water, acid, and pepsin remained substantially unaltered under the in-

TABLE V

Effect of intravenous infusions on concentration of hydrogen ion and pepsin in gastric juice

Substance infused	Control period	During hormone infusion				After infusion
	3 hr.	0–2 hr.	–4 hr.	–6 hr.	–8 hr.	+2 hr.
	Mean H^+ concentration (*mEq./liter*)					
Vehicle	38	24	32	32	33	36
ACTH	37	33	29	33	34	40
Corticosterone	38	30	46	38	41	44
Hydrocortisone	44	40	45	41	40	39
Prednisolone	22	22	27	36	39	
Aldosterone	22	37 (1 hr.)	41 (2 hr.)	56 (3 hr.)	51 (4 hr.)	41
Histamine	15	96	110	121	104	
	Mean pepsin concentration (*P.U./ml.*)					
Vehicle	1,233	1,262	1,430	1,639	1,494	1,521
ACTH	1,013	1,018	1,062	1,127	900	970
Corticosterone	1,418	1,377	1,641	1,626	1,700	1,571
Hydrocortisone	1,206	1,332	1,217	1,200	1,067	1,067
Prednisolone	1,083	1,138	1,576	1,562	1,147	
Aldosterone	944	2,310 (1 hr.)	2,200 (2 hr.)	2,265 (3 hr.)	2,036 (4 hr.)	1,306
Histamine	936	1,289	1,084	1,085	1,272	

TABLE VI

Effect of intravenous infusions on viscosity of gastric juice

Substance infused	No. of studies	Mean viscometer readings (*seconds*±*S.D.*) (*Water 7.35*±*0.05 sec.*)					
		Control	During infusion				After infusion
		3 hr.	0–2 hr.	–4 hr.	–6 hr.	–8 hr.	+2 hr.
Vehicle	13	8.59±3.2	8.32±1.6	8.05±1.3	8.30±1.4	8.44±1.6	8.06±1.3
ACTH	12	8.97±2.2	8.14±.75	8.15±.69	7.95±.64*	7.82±.55†	7.71±.55†
Corticosterone	9	8.38±.92	8.35±1.78	7.96±.12	7.94±.54	7.88±.27*	8.14±.61
Hydrocortisone	11	8.67±1.31	8.72±2.6	8.04±1.0	8.55±2.8	8.13±1.2	8.08±1.2
Prednisolone	2	8.50	8.47	8.44	8.09	7.80	
Aldosterone	1	8.46	8.82	7.92	8.28	7.92	7.68

* $p < 0.05$
† $p < 0.01$
Significance of decrease from control value by Student's 't' test. Other differences not significant.

fluence of the 8-hour infusion of ACTH or adrenocortical steroids. However, there were some significant changes in composition of the gastric juice.

Concentrations of acid and pepsin (Table V)

There was essentially no difference between the concentrations of acid in any of the hormone experiments and in their own control periods or in

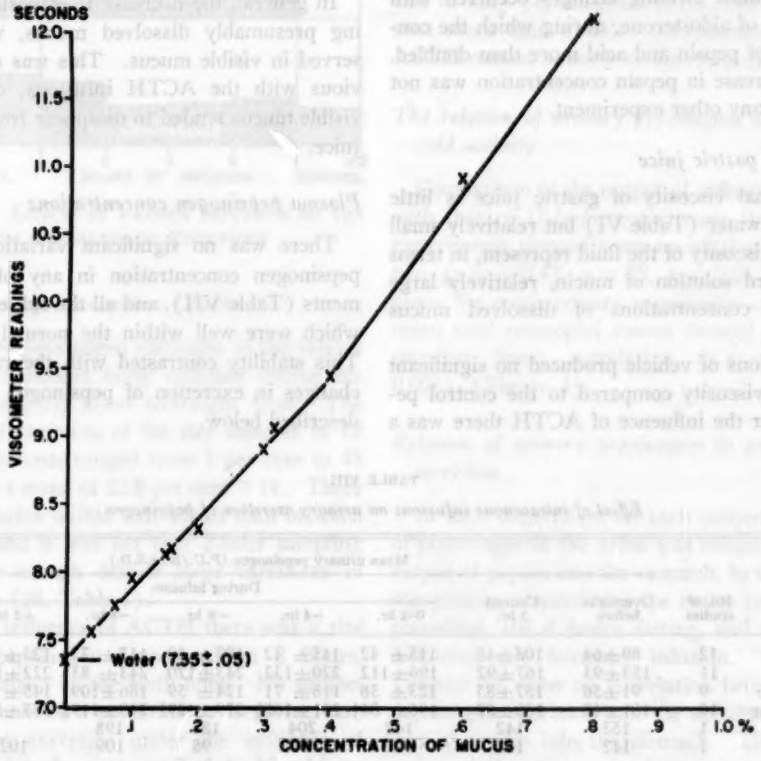

FIG. 1. VISCOMETER READINGS OF INCREASING CONCENTRATIONS OF WILSON HOG STOMACH MUCIN (1701-W) IN 0.04 N HCl, IN THE RANGE OF OBSERVED GASTRIC JUICE VISCOSITY

TABLE VII

Effect of intravenous infusions on concentration of pepsinogen in plasma

Substance infused	No. of studies	Mean pepsinogen concentration (*P.U. per 100 ml.* ±*S.D.*)						
		Control period		During infusion				After infusion
		−3 hr.	0 hr.	2 hr.	4 hr.	6 hr.	8 hr.	+2 hr.
Vehicle	13	12.3±2.4	11.6±3.0	12.6±3.2	13.7±5.4	12.8±3.4	11.6±3.5	14.0±5.1
ACTH	13	12.1±4.9	12.8±5.0	14.2±6.2	12.8±4.8	11.7±5.0	10.6±5.1	11.0±5.8
Corticosterone	9	12.3±2.6	13.2±3.2	16.7±4.7	13.6±3.0	10.5±3.8	11.7±5.3	16.2±7.5
Hydrocortisone	11	12.1±3.3	14.5±2.8	11.2±3.8	12.2±2.4	12.4±3.4	13.5±5.2	12.7±2.3
Prednisolone	2	7.5	7.5	7.8	7.1	7.1	7.8	
Aldosterone	1	7.9	8.1	8.7	9.3	8.7	8.9	10.3

the vehicle experiments. With infusion of the vehicle alone, the concentration of pepsin tended to rise as the experiment progressed. There was a persistent but smaller rise in the concentration of pepsin with the infusion of corticosterone, and no essential changes in concentration with the infusion of ACTH or of hydrocortisone or prednisolone. The most striking changes occurred with the infusion of aldosterone, during which the concentrations of pepsin and acid more than doubled. Such an increase in pepsin concentration was not observed in any other experiment.

Viscosity of gastric juice

The normal viscosity of gastric juice is little higher than water (Table VI) but relatively small changes in viscosity of the fluid represent, in terms of a standard solution of mucin, relatively large changes in concentrations of dissolved mucus (Figure 1).

The infusions of vehicle produced no significant changes in viscosity compared to the control period. Under the influence of ACTH there was a progressive fall in viscosity which became manifest in the first 2 hours of infusion, statistically significant by the sixth hour, and highly significant in the following 4 hours. There was a general tendency for the viscosity to fall in the other experiments, but only in the last 2 hours of the corticosterone infusion was this fall statistically significant.

In general, the decrease in viscosity, representing presumably dissolved mucus, was also observed in visible mucus. This was especially obvious with the ACTH infusions, during which visible mucus tended to disappear from the gastric juice.

Plasma pepsinogen concentrations

There was no significant variation in plasma pepsinogen concentration in any of the experiments (Table VII), and all the subjects had levels which were well within the normal range (25). This stability contrasted with the rather marked changes in excretion of pepsinogen in the urine, described below.

TABLE VIII

Effect of intravenous infusions on urinary excretion of pepsinogen

Substance infused	No. of studies	Mean urinary pepsinogen (*P.U./hr.* ±*S.D.*)							
		Overnight before	Control 3 hr.	During infusion					Overnight after
				0–2 hr.	−4 hr.	−6 hr.	−8 hr.	+2 hr.	
Vehicle	12	89±64	103±48	115± 42	115± 42	107± 40	117± 73	123± 74	
ACTH	11	153±93	167±92	196±112	220±132	243±120	243± 83†	222±108	148±54
Corticosterone	9	91±56	137±83	123± 58	118± 71	124± 59	186±109	145± 87	106±73
Hydrocortisone	10	101±48	130±57	196± 84†	251±165‡	379±429†	248±119‡	255±107‡	171±79
Prednisolone*	1	153	142	142	204	182	198		
Aldosterone*	1	142	148	142	99	98	109	102	152

* Same subject.

† $p < 0.05$
‡ $p < 0.01$ } Significance of increase over control period. Other differences not significant.

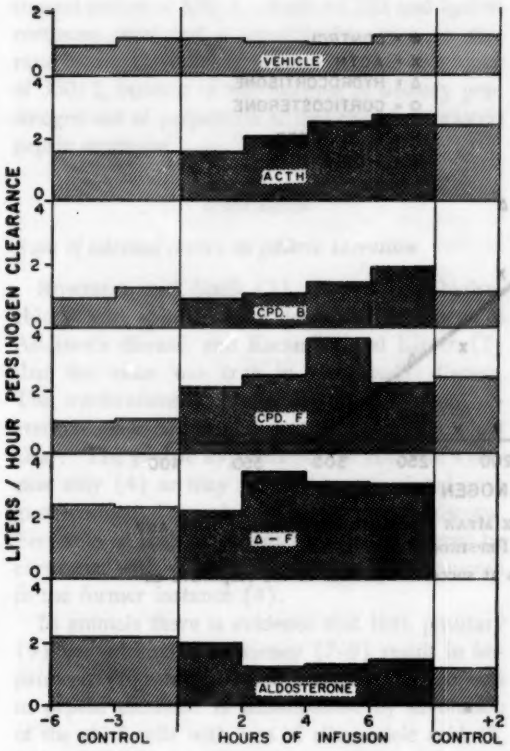

FIG. 2. MEAN EFFECTS OF VARIOUS INFUSIONS ON THE RENAL CLEARANCE OF PEPSINOGEN

Urinary pepsinogen excretion (Table VIII)

The urinary pepsinogen excretion in the vehicle experiments remained fairly constant during the day, with a slightly lower overnight level. The coefficients of variation of the day samples in 12 vehicle experiments ranged from 1 per cent to 45 per cent with a mean of 22.8 per cent ± 14. There was less variation within individuals than between individuals, and it was felt that 2-hour sampling was accurate enough despite large variations in urine volume (26, Table I).

Under the influence of ACTH there was a rise in urinary pepsinogen output within 2 hours, reaching a plateau by the sixth hour. No subject failed to experience a moderate to large increase in pepsinogen excretion under the influence of ACTH. With hydrocortisone 2 of the 10 subjects had no increase in urinary pepsinogen excretion. However, the mean response to the infusions of hydrocortisone was a statistically significant increase over the control period, from the second hour of the hormone infusion until the 2-hour period after the completion of the infusion. With corticosterone, 5 of the 9 subjects showed a small increase in pepsinogen excretion, but the mean showed no significant increase for the group over the control period. With aldosterone there was a drop in urinary pepsinogen excretion, and with prednisolone an increase in the one experiment where urinary pepsinogen was measured. The effects of hydrocortisone persisted during the night after the infusion, whereas the effects of the other hormones did not continue for more than 2 hours after their infusion.

The clearance of pepsinogen

The calculated renal clearances of pepsinogen indicate, as would be expected from the plasma and urinary pepsinogen figures, that hydrocortisone more than doubled the clearance, and that ACTH caused a moderate increase (Figure 2).

The relation of urinary pepsinogen to glucocorticoid activity

Comparison of the output of urinary pepsinogen with that of 17-hydroxycorticoids in the ACTH experiments showed some association between the two ($r = +0.43$, $n = 55$, $p < 0.01$). Furthermore, the mean urinary pepsinogen excretion and mean total eosinophil counts showed an excellent negative linear correlation ($r = -0.88$, $p < 0.001$), (Figure 3).

Relation of urinary pepsinogen to gastric pepsin secretion

In each experiment on each subject the output of pepsinogen in the urine was compared with the output of pepsin into the stomach, in three periods comprising, respectively, the entire control period preceding, the 8 hours during, and the 2 hours following the hormone infusion. These data (Figure 4) show no correlation between urinary pepsinogen excretion and the simultaneous secretion of pepsin into the stomach. The calculated ratios of Gastric pepsin : Urinary pepsinogen for these periods showed very wide fluctuations ranging from 88 : 1 to 2,650 : 1 with a mean ratio in the

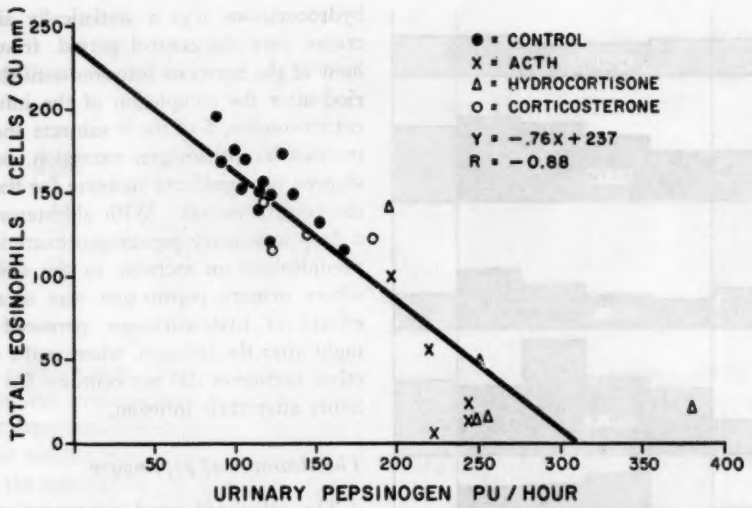
TOTAL EOSINOPHILS (CELLS / CU mm)
URINARY PEPSINOGEN PU / HOUR
● = CONTROL
X = ACTH
Δ = HYDROCORTISONE
○ = CORTICOSTERONE
Y = −.76X + 237
R = −0.88

FIG. 3. NEGATIVE CORRELATION BETWEEN THE MEAN ABSOLUTE EOSINOPHIL COUNT AND SIMULTANEOUS URINARY PEPSINOGEN EXCRETION

Each coordinate represents the mean values at successive periods in the respective experiments (see Tables I and VIII).

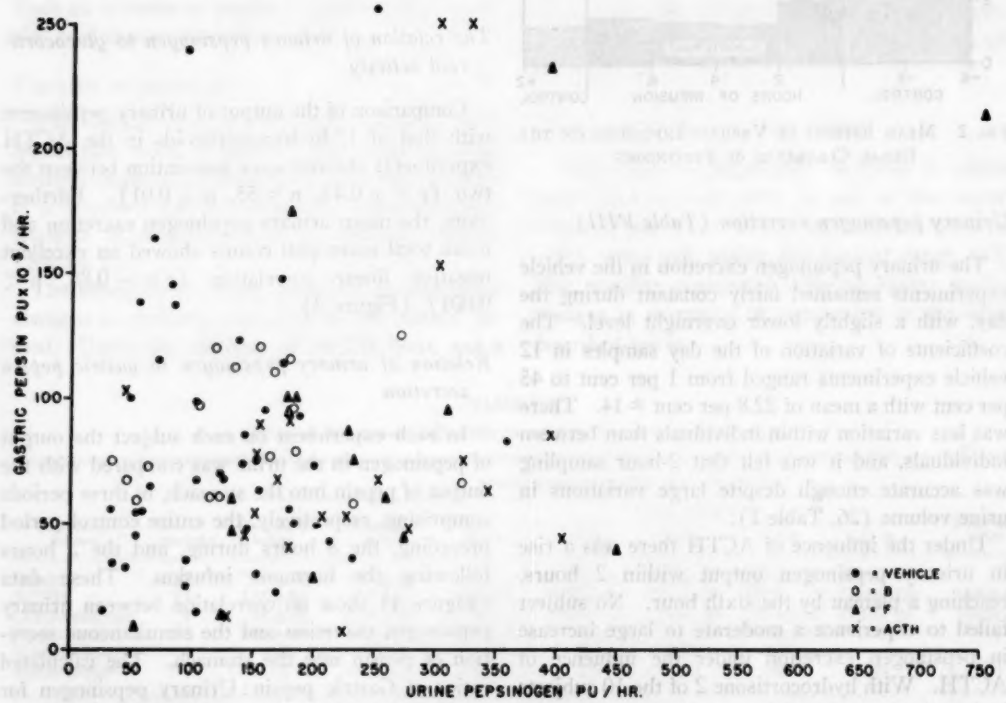

FIG. 4. LACK OF CORRELATION BETWEEN THE OUTPUT OF PEPSIN IN THE GASTRIC JUICE AND SIMULTANEOUS URINARY PEPSINOGEN EXCRETION (SEE TEXT)

control period of 830:1. Both ACTH and hydrocortisone produced a considerable drop in this ratio during the 8 hours of their infusion to a mean of 350:1, because of the increase in urinary pepsinogen out of proportion to any change in gastric pepsin secretion.

DISCUSSION

Role of adrenal cortex in gastric secretion

Rowntree and Snell (1) showed that hydrochloric acid secretion was depressed or absent in Addison's disease, and Escamilla and Lisser (2) that the same was true in Simmonds' disease. The mechanisms whereby the adrenocortical secretions maintain normal gastric secretion are not clear. The gastric hypofunction in Addison's disease may (4) or may not (3) be associated with gastric mucosal atrophy as judged by gastroscopy. Secretion in both cases was restored to normal by cortisone, with a return to normal of the mucosa in the former instance (4).

In animals there is evidence that both pituitary (9) and adrenal insufficiency (7–9) result in impairment of gastric secretion, and that the decrease in pepsin secretion is accompanied by involution of the chief cells with loss of ribonucleic acid (8, 9). Confirmation of this involution, noted histologically, was obtained by chemical analysis for pepsinogen which was decreased in the mucosa of the stomachs of adrenalectomized rats (11). Restoration of secretion with adrenal cortex extract (7) and of normal synthesis of pepsinogen by cortisone, corticosterone, hydrocortisone, and desoxycorticosterone, as well as by cortical extract (11), indicates the relatively nonspecific steroid requirements of the chief cells for normal function. It would thus appear that the adrenocortical secretions are necessary to maintain both structural and functional integrity of the gastric mucosa, probably acting in a permissive (6) or trophic role.

On the other hand, the evidence in support of a stimulant role for the adrenal cortex in gastric secretion is both inadequate and conflicting. Gray and his colleagues have claimed such a role for the adrenal both in acute (13) and in chronic experiments (12, 27, 28). In the published evidence presented by these authors many of the values for gastric juice volume, acid and pepsin are within the limits of fluctuation in the basal state, and where significant increases were noted, these were usually in the second or third week of sustained high adrenocortical activity. The observations of these authors are supported by the finding in four dogs of a moderate, though inconsistent, increase in acid output while the animals were being given large doses of ACTH (14), and by unconvincing evidence of hypersecretion of acid in Cushing's syndrome (5). Moreover, much of the evidence for an increase in gastric secretion produced by ACTH and the adrenocortical steroids is indirectly derived from the increase in *urinary* pepsinogen excretion under the influence of these hormones (27, 28). As has been shown before (15, 29) and again in this paper, the rise in urinary pepsinogen is not a reflection of similar changes in gastric secretion. In contrast with the findings of Gray and his associates (12, 13, 27, 28), Kirsner and Ford (16) have recently reported that ACTH and cortisone administration over prolonged periods failed to increase gastric secretion in man, and Davenport and Chavré (30) failed to show any stimulant effects of cortisone on their *in vitro* preparations of mouse stomach. The present results have shown no evidence of any significant stimulation of the secretion of acid or pepsin by the infusions of ACTH, hydrocortisone or corticosterone. In order to place in their proper physiological perspective the effects of the hormone infusions on gastric secretion, the very pronounced effects of intravenous infusions of histamine have been described alongside those of the hormones. It is possible that the hormone infusions were too short to have caused any great changes in gastric secretion, though Villarreal, Ganong, and Gray (13) have described increases in gastric secretion within 4 to 6 hours of ACTH administration in dogs. Moreover, 5 subjects receiving ACTH-gel for 6 days (15) showed only small and inconsistent changes in gastric secretion, while 4 subjects receiving 150 mg. hydrocortisone orally daily for 20 to 30 days and two others, receiving 100 mg. cortisone acetate orally daily for 40 days showed no increase in acid or pepsin secretion in basal gastric secretion, determined twice weekly (18).

The composition of gastric juice

The principal change in the gastric juice observed in these experiments was a decrease in

viscosity in subjects receiving ACTH or corticosterone. Hydrocortisone and prednisolone had no significant effect on viscosity. These results suggest that the effect of ACTH on viscosity was not mediated by hydrocortisone, and may have been mediated by corticosterone or by some other hormone or contaminant. A similar decrease in viscosity has been seen previously with more prolonged ACTH administration in man (15), and in dogs receiving ACTH (13). In adrenalectomized rats the viscosity of gastric juice is much increased (17), and hydrocortisone or cortisone produce a decrease of stainable mucus on the surface and in the pits of the gastric mucosa of rats (10). These results suggest an important but still unclear role of the adrenal cortex in the regulation of gastric mucus secretion.

With regard to the concentration of acid, there was no striking effect of ACTH, hydrocortisone or corticosterone. The one experiment in which aldosterone was given showed a fairly steep increase in H ion concentration. Of all the experiments, only the one with aldosterone had a clear-cut stimulant effect on the concentration of pepsin in the gastric juice. While it would be unwise to draw sweeping conclusions from one experiment, the data are striking enough to deserve consideration. We have recently suggested (11) that sodium depletion in rats (in the face of adrenalectomy) results in gross depression of the *synthesis* of gastric pepsinogen. The apparently marked stimulation of gastric pepsin secretion by aldosterone, the most potent of the salt-retaining hormones, suggests that sodium may well play an important part in the secretion of pepsin as well as in its synthesis.

It is not surprising that many of the effects of ACTH, notably eosinopenia and urinary pepsinogen increase, were reproduced by hydrocortisone, in view of the release of a preponderance of this steroid from the human adrenal cortex stimulated by ACTH (25). On the other hand, not all of the effects of ACTH could be reproduced by hydrocortisone. The fall in gastric juice viscosity, for instance, was not observed in the hydrocortisone experiments, but did occur in the corticosterone experiments. This observation may have identified one physiological role for corticosterone in man.

The control and significance of urinary pepsinogen excretion

This study has confirmed that ACTH invariably increases the excretion of urinary pepsinogen and that such increases occur within a few hours of the start of the ACTH administration. It has also indicated that such increases did not reflect similar changes in gastric secretion. The evidence further shows that the changes observed with ACTH were the result of glucocorticoid activity, acting principally, if not wholly, on the kidney. The significant role of the glucocorticoids in the regulation of urinary pepsinogen excretion, found in these experiments, is supported by the positive correlations between urinary pepsinogen and urinary 17-hydroxycorticoids in Addison's disease, in Cushing's syndrome, and during ACTH stimulation (31).

The amount of pepsinogen excreted in the urine depends on at least three factors (29). Since the stomach is the source of urinary pepsinogen, the gastric peptic cell mass (21) in part determines whether there is none in the urine, as in pernicious anemia (32, 33) or an increased amount as in duodenal ulcer (32, 33), and especially so in long-standing duodenal ulcer (22), where the gastric peptic cell mass can reasonably be assumed to be increased.

The second factor involved is the secretion gradient of the peptic cells, that is, the proportion of the peptic cell pepsinogen which is secreted into the stomach, as compared with that passing into the blood. We have recently observed two cases (15, 34) in which the secretion gradient was reversed and a large proportion of the gastric cell pepsinogen passed into the blood and so into the urine, resulting in great increases in urinary pepsinogen, while the amount secreted into the stomach was strikingly reduced. The reasons for and mechanisms of this reversal are not known.

The third factor involved is the renal clearance of pepsinogen from the plasma. Under both the conditions mentioned above, the clearance of pepsinogen was normal (29) and the increases in urinary pepsinogen resulted directly from the elevated levels of plasma pepsinogen. On the other hand, the increases related to adrenal glucocorticoid activity occurred with unchanged plasma pepsinogen levels and little or no change in gastric

pepsin secretion, and were due entirely to increases in pepsinogen clearance by the kidney.

A high level of urinary pepsinogen may therefore be found with a high, low, or normal amount of gastric pepsin secretion, depending on the particular set of circumstances under which it is measured. Conversely, there may be an increased amount of pepsin secreted into the stomach with a normal, or, less commonly, a decreased amount of pepsinogen excreted into the urine.

The lack of correlation between gastric pepsin and urinary pepsinogen found in this study may appear to differ strikingly from the positive correlation reported by Janowitz and Hollander (32). When the normal group included in their report is examined alone, however, there is little if any direct correlation between urinary and gastric pepsinogen. Their over-all correlation coefficient is weighted by the cases with pernicious anemia at one extreme and some of the cases with duodenal ulcers (increased peptic cell mass) at the other extreme.

SUMMARY

In 49 experiments on 16 normal subjects, the effects of 8-hour intravenous infusions of ACTH, corticosterone, hydrocortisone, aldosterone and prednisolone on gastric secretion and on plasma and urine pepsinogen were compared with values found in the preceding 3-hour control period and with experiments in which no hormone was infused. The gastric effects of these hormone infusions were contrasted with 10 experiments in which histamine was infused for 4 hours.

No changes in concentration or output of acid or pepsin could be attributed unequivocally to the hormone infusions, except for a doubling of the acid and pepsin concentrations during the single infusion of aldosterone. The viscosity of the gastric juice was significantly reduced by ACTH and by corticosterone, but not by hydrocortisone, suggesting one possible role for the corticosterone which is secreted by the human adrenal.

ACTH invariably increased the output of urinary pepsinogen while the plasma pepsinogen levels remained unaltered, the increases being due entirely to an increased renal clearance of pepsinogen. These effects were reproduced by hydrocortisone and to a much smaller extent by corticosterone, while aldosterone had the opposite effect. An excellent negative correlation between the mean eosinophil count and the amount of pepsinogen in the urine indicated that the normal variations of pepsinogen in the urine were probably under control of the glucocorticoids. The reasons for the lack of correlation between gastric pepsin and urinary pepsinogen lie in the complex and ill-understood factors which govern the discharge of the enzyme from the gastric cells into the blood and in the secondary factors which determine the amount of the enzyme cleared from the blood by the kidney.

ACKNOWLEDGMENTS

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STUDY OF URINARY AND FECAL EXCRETION OF RADIOACTIVE CHROMIUM Cr^{51} IN MAN. ITS USE IN THE MEASUREMENT OF INTESTINAL BLOOD LOSS ASSOCIATED WITH HOOKWORM INFECTION [1]

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Since the work of Cruz (1) and Rhoads, Castle, Payne, and Lawson (2) iron deficiency has been attributed a pre-eminent role in the causation of the anemia associated with hookworm infection. At one time or another, however, this anemia has been blamed on the effect of hemolytic "toxins" from the hookworm, poor iron absorption, poor protein intake, and blood loss, or a combination of all or several of these factors. An exact evaluation of the role of each of these possible causes is not feasible until quantitative studies are carried out in the human being infected with the human hookworm.

In the present study, quantitative evaluation of one of these factors, namely blood loss due to the parasite, is attempted.

The radioactive isotope of chromium Cr^{51} has been used for marking red blood cells (3, 4), estimating their life span and measuring blood volume (4, 5). As a preliminary to our investigation on intestinal blood loss produced by hookworm, we wished to know: 1) How much Cr^{51} is absorbed from the intestinal tract when it is introduced into the stomach or duodenum of normal or hookworm infected patients, 2) whether Cr^{51} which is absorbed from the intestinal tract enters the circulating erythrocytes in significant quantities, 3) what proportion of Cr^{51} within the circulating red blood cells appears daily in the feces and urine of non-infected subjects.

In the present report, it is shown that most of the Cr^{51} from tagged circulating erythrocytes is excreted via the urine, that practically negligible amounts of Cr^{51} introduced into the gastrointestinal tract are absorbed, and that this absorbed Cr^{51} does not enter erythrocytes in measurable quantities. Furthermore, it appears from this study that blood loss, although less than the loss due to *A. caninum* in the dog (6, 7), is probably an important factor in the genesis of the anemia associated with human hookworm infection.[2]

METHODS

All subjects were hospitalized in the Medical or Surgical wards of the Hospital Vargas. Except for patients L.M. and J.F. (Table II), the non-infected subjects were patients who had been hospitalized for surgery, or for local skin conditions. In all subjects in this category, feces were free from hookworm ova in at least three examinations. Patient L.M. was a 30-year-old woman who had had an acute hemorrhage due to placenta previa, and was studied from day 13 to day 25 post partum. Patient J.F. had acute leukemia.

Tagging of circulating erythrocytes. Approximately 20 ml. of blood was taken from the antecubital vein and introduced into a sterile bottle containing 3 ml. of A.C.D. solution and approximately 80 microcuries of Cr^{51}.[3] The mixture was incubated at 37° C. and gently shaken every five minutes. The blood was then washed three times with 0.9 per cent saline solution in experiments in which intestinal absorption was studied, or reintroduced into the patient's circulation without further washing in the studies of Cr^{51} excretion from circulating erythrocytes.

Study of Cr^{51} absorption from the gastrointestinal tract. The washed erythrocytes were resuspended in 5 per cent dextrose. A Levine tube was placed in the patient's duodenum, under fluoroscopic control, and exactly 10 ml. of the suspension was introduced through the tube by gravity with a volumetric pipette, followed by six successive washings with 10 ml. of the diluting fluid. After thorough cleaning and decontamination of the pipette, a standard was prepared for each patient with the same

[1] This work was supported by a grant from the Creole Petroleum Corporation.

[2] A preliminary note on this work has appeared (8). The discrepancies between the figures in the note and those in the present communication are due to the fact that at the time it was not realized that five of the cases were mixed *Ancylostoma-Necator* infections.

[3] From Abbott Laboratories, Oak Ridge, Tennessee.

pipette and in the same way used for intraduodenal administration and the washings were diluted up to 500 or 1,000 ml. with water. For the experiments in which sodium radio chromate in water was used, the dissolved radioactive substance was measured out volumetrically in a glass. The solution was then drunk by the patient, followed by six successive rinsings. Stools, 24-hour urine and venous blood were obtained daily, the radioactivity determined, and compared to radioactivity in the standard. The results were expressed in terms of per cent of the administered Cr^{51}, except for the activity of blood, in which results were expressed in terms of counts per minute per ml. of blood. Measurements were carried out until no more activity appeared in the feces.

Study of excretion of Cr^{51} from tagged circulating erythrocytes. After waiting for a period of at least four days after tagging of the circulating erythrocytes, stool and urine collections were begun. Stools were obtained in four-day periods in wide mouthed jars lined with aluminum foil into which the patients defecated directly. The stools were weighed to the nearest gram and homogenized in a Waring blendor with measured quantities of water. An aliquot of the homogenate was introduced with a special applicator into the bottom of a test tube, so that the upper level of the sample reached approximately a 3-ml. calibrated mark on the tube. The fecal sample was weighed by difference to the nearest 0.1 mg. and the result corrected for the proportion of water introduced in the stool. The radioactivity of the fecal aliquot was compared to that of 3 ml. of blood obtained from the patient at the start of the collection period, four days previously. The results were expressed in terms of ml. of blood with a radioactivity equivalent to that found in the stools during the period of study. This value was obtained, for a four-day period, by the formula:

$$\frac{R_s \times Q}{R_b \times 4} = B \qquad (1)$$

in which,

R_s = Radioactivity in the four-day stool in counts per minute per gram of stool.

R_b = Radioactivity in the blood of the same patient obtained at the beginning of the four-day stool collection in counts per minute per ml. of blood.

Q = Quantity of stool in the four-day period, in grams.

B = Quantity of blood in ml. which contains the amount of radioactivity found in the stools in 24 hours.

The average of the studies from three such four-day periods is given for each subject. Patient J.F. (Table II) was studied, of necessity, in a somewhat different manner, his circulating erythrocytes having been tagged on day 0, and stool collections begun on day 4. As the patient was in poor condition and ate little, no feces were passed until day 10, shortly before death. At autopsy, performed 24 hours later, the contents of the small intestine and colon were collected and their radioactivity determined. It was reasoned that the total amount of radioactivity present in the feces, small intestine and colon represented the total amount of Cr^{51} which had been excreted into the gastrointestinal tract since stool collections had begun.

The use of the blood radioactivity at the start of a four-day period of stool collection as a standard for comparing with stool radioactivity during the subsequent four days was to some extent arbitrary. Ideally, the standard blood value to compare with fecal radioactivity should be the average blood radioactivity during the time in which Cr^{51} present in the four-day stools is being excreted into the intestinal tract. This would imply a knowledge of the intestinal transit time for each individual patient during the time of experiment and intestinal site at which Cr^{51} is being excreted. As it was not possible to obtain this value, the above compromise was used.

Urine radioactivity was measured in 3 ml. of undiluted urine, and the activity compared to that in 3 ml. of blood obtained on the same day. The results were expressed as for the stools. Urine was collected in three 24-hour samples and the results given are the average of three determinations.

Plasma volume was measured by the Evans blue method (9) and the blood volume calculated from the venous hematocrit multiplied by 0.9 (10), to correct for differences between venous and body hematocrit. The hematocrit was obtained by centrifuging the blood for 30 minutes at 3,000 revolutions. No correction for trapped plasma was used. Thus the value B, in formula (*1*) could be expressed in terms of per cent of the blood mass which contains the amount of radioactivity found in the stools in 24 hours.

Measurement of radioactivity. Radioactivity was determined in a well-type scintillation counter (Nuclear, model 3037-B). By mixing a water solution of radioactive sodium chromate of known radioactivity with stools of varying consistency, it was repeatedly shown that under the present experimental conditions there was no significant self-absorption. In Table I are shown the results of such an experiment; approximately 0.9 μc. of Cr^{51} was mixed with 1,000 ml. of water; a specimen of non-radioactive hard feces was chosen and mixed with chromated water in the proportions, by weight, shown in Table I. As may be seen, only when feces were mixed with water in proportion 2:1 was there any apparent reduction of the expected count, by 2.7 per

TABLE I

Effect on measured radioactivity of mixing Cr^{51} in water with hard feces

Dilution Cr^{51} in water/feces	Radioactivity Cr^{51} in water alone	Radioactivity Cr^{51} in feces	% Difference
	c.p.m./ml. of water	*c.p.m./ml. of water*	
1:2	7,058	6,866	−2.7
1:1	7,058	7,056	0.0
1:1	7,058	7,149	+0.1
2:1	7,058	7,177	+0.3

cent. In these experiments, a minimum of 20,000 counts was determined. In all other dilutions, and in experiments in which semi-soft, normal stools were used, there was no detectable self-absorption. Consequently, in our studies of fecal radioactivity, when a hard stool was obtained, a dilution of at least 1 : 1 was used.

For the sake of convenience, the quantity of blood in ml. which contains the amount of radioactivity found in the stools or in the urine in 24 hours is expressed hereafter as "ml. of blood per 24 hours" in the stools and in the urine, respectively. A minimum of 6400 counts was measured on each sample.

Studies on hookworm-infected subjects. All subjects in this study were also hospitalized in the wards of the Hospital Vargas. Most of the patients with moderate or no anemia had been hospitalized for different unrelated complaints and were found incidentally to pass hookworm ova in their feces. Those with marked anemia came in with complaints related to the anemia.

Stool collections, marking of the circulating erythrocytes and determination of radioactivity were done as for non-infected patients. Hemoglobin was determined by the cyanomethemoglobin method (11). Ova counts were done by the method of Caldwell and Caldwell (12).

At the end of the initial 12-day stool study,[4] each patient was given 3 ml. of tetrachlorethylene by mouth, followed in two to three hours by a magnesium sulphate cathartic. During the ensuing days, stools were collected in 24-hour periods and washed repeatedly with tap water in a fine-meshed cloth bag until a thoroughly homogenized residue was obtained. The hookworms were then sought and picked out individually until no more worms could be found. The worms were placed in 70 per cent alcohol or 10 per cent formalin solution in a test tube. In some of the cases, the test tube was placed in the well-type scintillation detector and radioactivity determined. Stools were collected until they were found to be free from hookworms. Stool radioactivity was again studied during three successive four-day periods.

After gentle and thorough shaking of the hookworms in the test tube to insure uniform distribution, a representative sample of at least 100 worms was examined (or the whole sample when there were less than 100 worms). Each worm was cleared in creosote under a coverslip and identified under the microscope. Although it has been thought that *Ancylostoma* duodenale infection did not exist in Venezuela (13), five of the 20 cases examined were found to harbor this parasite, along with *Necator americanus* (14).

In some patients, in whom a good number of ova remained after the first vermifuge, tetrachlorethylene was administered a second time, and a third twelve-day study period was carried out.

The blood was "marked" again when radioactivity decreased below 100 counts per minute per ml. above background. The quantity of blood lost per hookworm per day was calculated from

$$H = \frac{A - B}{N} \qquad (2)$$

in which

A = Average amount of blood in the stool in ml. per day, determined from a 12-day study period before the administration of vermifuge.
B = Average amount of blood in the stool in ml. per day determined in the same way after the administration of tetrachlorethylene.
N = Number of hookworms recovered from the stools after the vermifuge given between periods A and B.

In order to attempt to establish a relationship between oviposition and blood-sucking activity of the worms, the blood loss per one hundred thousand ova per day was similarly calculated.

Since there were no pure *Ancylostoma* infections, a rough estimate of the blood loss occasioned by *Ancylostoma* was attempted by assuming that the *Necator* present were responsible for an intestinal blood loss calculated from the average found in pure *Necator* infections. Then,

$$B_a = \frac{100\,B_h - (3.11 \times 10^{-3}\,NH)}{AH}$$

in which

B_a = Blood loss per *Ancylostoma*, in ml. per day.
B_h = Total blood loss due to hookworms (*Necator* + *Ancylostoma*) as calculated by formula (*2*).
3.11×10^{-3} = Average blood lost per *Necator* (Table VI) in ml. per day.
N = Per cent *Necator* in the sample studied.
A = Per cent *Ancylostoma* in the sample studied.
H = Total number of hookworms (*Necator* + *Ancylostoma*) recovered.

An estimate of iron lost into the gastrointestinal tract was made by applying the formula:

$$Q_{Fe} = \frac{Hb \times B \times 3.40}{100}$$

in which

Q_{Fe} = Mg. of iron lost per day into the gastrointestinal tract.
Hb = Blood hemoglobin in Gm. per 100 ml.
B = Ml. of blood lost per day in the intestine.
3.40 = Mg. of iron per Gm. of hemoglobin.

Gastrointestinal studies, including X-ray visualization of esophagus, stomach, duodenum, small intestine and colon, and sigmoidoscopy were performed on patients 1 to 6, 11, 14, and 21. These revealed no source of bleeding. Patient 9 was autopsied and no source of bleeding other than hookworm infection was found.

RESULTS

The results are shown in Tables II to VII. In Tables III and IV, blood radioactivity is not tabulated, since in all cases except one it was not above background. In the case of M.P., Table IV, the

[4] Except on patient 1, who was studied for six days only because of his poor condition.

TABLE II

Fecal and urinary excretion of Cr^{51} tagged onto erythrocytes in non-infected patients

Subject Sex	Diagnosis	Feces		Urine		Blood hemoglobin
		ml. of blood/day	*% of blood mass/day*	*ml. of blood/day*	*% of blood mass/day*	*Gm./100 ml.*
J. S. M	Cutaneous leishmaniasis	0.71	1.22×10^{-2}	24.85	0.43	15.2
A. R. M	Chromoblastomycosis	1.05	2.03×10^{-2}	38.80	0.75	15.4
J. M. M	Cutaneous leishmaniasis	1.76	5.74×10^{-2}	49.09	1.40	14.4
J. R. M	Erythema induratum (Bazin)	1.04	2.01×10^{-2}	32.91	0.64	13.8
L. O. F	Bronchopneumonia (cured)	1.54	3.45×10^{-2}	28.33	0.59	12.5
M. B. F	Chronic cholecystitis	0.28		37.53		12.1
A. A. F	Erythema multiforme	2.14	6.72×10^{-2}	28.30	0.89	13.8
V. S. F	Cutaneous leishmaniasis	1.79	4.50×10^{-2}	26.82	0.67	14.0
L. M. F	Placenta previa-acute hemorrhage	0.42				6.1
J. F. M	Acute leukemia	1.98				4.2
Average		1.27	3.67×10^{-2}	33.33	0.77	
Range		0.28 2.14	1.22×10^{-2} 6.72×10^{-2}	24.85 49.09	0.43 1.40	

blood counted consistently 14 counts per min. per ml. above background throughout the period of study. In Table V, the patients are arranged according to the quantity of blood loss, which is roughly that of decreasing severity of infection, as judged from the total number of hookworms recovered and the ova remaining in the stools after vermifugation. In column 1 are shown the average of the first three ova counts in each patient. In column 2, the number of hookworms is the total number recovered after one or two vermifuges. In patients 9, 10, 12 and 21, recovery of the hookworms was not attempted. Column 3 shows the blood hemoglobin value upon admission to hospital and column 4 at the time of study. When values in columns 3 and 4 coincide, the patient was studied approximately during the first two weeks after admission, and the two similar values are one and the same determination. In column 5, the values represent the average of three successive four-day fecal samples. In patients 7, 10, 11, 13, 14, 15, 17 and 19, blood loss was not studied on admission, but only after partial recovery from anemia or, in case 8, after reduction of the blood hemoglobin, presumably because of bleeding due to hookworms. In Table VI, the number of hookworms is that recovered after the first vermifuge only, between periods A and B. In the column marked "patient" the numbers 1 and 2 in parentheses indicate two different studies in the same patient, before and after the first vermifuge, and before and after the second vermifuge, respectively. Thus there are fourteen studies in twelve patients.

DISCUSSION

Owen, Bollman and Grindlay (15) have shown in two dogs that most of the Cr^{51} tagged onto erythrocytes introduced into the stomach of these animals is recovered in the feces, and that most of the excreted radioactivity from Cr^{51} in circu-

lating erythrocytes appeared in the urine. In this respect, man seems to be similar to dog. On the other hand, in one dog who was given an aqueous solution of radiochromate by stomach tube, nearly half of the Cr^{51} was absorbed, whereas in our four subjects (Table IV) there seemed to be very little absorption of Cr^{51} under the same conditions. It has been suggested (15) that marking circulating erythrocytes with Cr^{51} and studying stool and blood radioactivity comparatively would be a means of determining the exact quantity of blood loss from gastrointestinal lesions. The same authors later reported two studies of intestinal bleeding in human patients (16), but they do not appear to have determined previously Cr^{51} excretion and absorption in the human. From our findings (Tables II, III and IV), it would seem that the method is a valid one in the human, since only negligible amounts of circulating Cr^{51} bound to erythrocytes (average 1.27 ml. of "blood" per day) are excreted in the feces (Table II) and since most of the radioactivity administered into the duodenum or stomach is recovered in the feces (Tables III and IV). Indeed, there seems to be little absorption of Cr^{51} even if this substance is given by mouth, either tagged on red cells or as sodium chromate in water (Table IV). It was felt that patient M.P. (Table IV) failed to collect feces on day 3, and that this accounted for the relatively low fecal recovery of 59.2 per cent, since the missing radioactivity was not recovered in the urine and since blood radioactivity was never above background in this patient.

Patients with marked anemia did not seem to differ from patients without anemia either with respect to intestinal absorption of Cr^{51} (Table III, patients M.R., J.V., and A.G.) or with respect

TABLE III

Per cent recovery in feces and urine of red-cell-attached Cr^{51} introduced into the duodenum

Patient Sex Presence of hookworm infection	Blood hemoglobin	Excretum studied	Per cent recovery* Day after administration 1	2	3	4	5	6	7	8	9	Total	Cumulative total
	Gm./100 ml.												
A. R.	15.4	Feces	0	—	95.3	—	2.0	—	0			97.3	97.6
M		Urine	0.3	0	0	0	0	0				0.3	
no													
J. S.	15.2	Feces	—	0.4	29.8	—	42.5	—	18.0	0.01		90.7	92.2
M		Urine	1.3	0	0	0	0	0	0	0.2		1.5	
no													
J. A.	15.2	Feces	4.7	50.8	24.3	11.2	0.1	0				91.1	91.5
M		Urine	0.1	0.1	0.1	0	0					0.4	
yes													
P. B.	12.4	Feces	32.6	58.9	11.8	0						103.3	105.7
M		Urine	0.9	0.8	0.7							2.4	
yes													
M. R.	5.9	Feces	67.5	25.4	12.1	—	0					94.5	99.8
F		Urine	4.1	0	0	0	1.2					5.3	
yes													
J. V.	6.2	Feces	84.2	14.7	0							98.9	98.9
M		Urine	0	0	0							0	
yes													
A. G.	5.4	Feces	0	83.5	19.0	1.0	0	0				103.5	105.3
F		Urine	1.3	0.3	0.2	0	0					1.8	
yes													
J. J. M.	11.5	Feces	0	0	0.8	90.8	—	2.1	—	0.5	0	94.2	
M		Urine	Not studied										
yes													
Average		Feces										96.7	98.7
		Urine										1.7	

* 0 = no radioactivity present in sample; — = no feces obtained.

TABLE IV

Per cent recovery in feces and urine of Cr^{51} given by mouth

Patient Sex Presence of hookworm infection	Blood hemoglobin	Substance administered*	Excretum studied	Per cent recovery† Day 1	2	3	4	5	6	7 to 13	14	Total	Cumulative total
	Gm./100 ml.												
A. P.	10.1	W	Feces	68.4	13.3	3.6	0.1	0.8	0			86.2	95.2
F			Urine	5.1	1.4	1.7	0.6	0.2	—			9.0	
no													
J. M.	14.4	W	Feces	87.8	—	6.6	3.4	0.2	0			98.0	99.8
M			Urine	1.3	0.3	0.2	—	—				1.8	
no													
L. R.	14.3	W	Feces	0	48.0	—	—	—	47.7	—	0.1 0	95.8	103.2
F			Urine	5.7	0.6	0.8	0.2	0.1	0	0	0	7.4	
no													
M. P.	13.7	W	Feces	3.9	2.7	—	52.6	0				59.2	60.2
F			Urine	0.6	0.3	0.1	0					1.0	
no													
S. B.	15.8	E	Feces	35.0	38.6	15.2	0					88.8	89.3
M			Urine	0.5	0	0						0.5	
yes													
B. M.	7.1	E	Feces	0	56.3	43.2	0.2	0				99.7	101.8
M			Urine	2.1	0	0	0	—				2.1	
yes													

* W = Aqueous solution of sodium chromate; E = Chromium-tagged, washed erythrocytes.
† 0 = No radioactivity present in sample; — = No feces obtained.

to fecal excretion of Cr^{51} (Table II, patients L.M. and J. F.).

Most of the radioactivity which escapes into the urine when Cr^{51} is administered into the gastrointestinal tract usually appears during the first 24 hours after administration of the radioactive material. Whether this means that absorption from the upper gastrointestinal tract is greater

TABLE V

Intestinal blood and iron loss in patients with hookworm infection

Column number Patient	Age	Sex	1 Hookworm ova	2 Total no. of hookworms recovered	3 Blood hemoglobin On admission	4 Blood hemoglobin At time of study	5 Fecal blood loss	6 Calculated iron loss
			1,000/day		*(Gm./100 ml.)*		*ml./day*	*mg./day*
1*	24	M	8,714	3,534	2.0	2.0	251.5	17.1
2	18	F	2,983	3,043	3.7	6.6	99.3	22.3
3	14	M	6,748	1,641	7.8	9.3	92.0	29.1
4	60	M	1,639	1,121	8.8	8.8	65.0	19.5
5	36	M	1,708	1,684	3.9	3.9	57.2	7.6
6	35	M	3,281	840	3.6	8.9	56.1	6.7
7*	24	F	1,824	258	3.1	11.4	49.8	19.3
8*	56	M	3,511	502	10.2	7.1	46.3	11.2
9	50	M	583		4.9	4.9	39.6	6.6
10	38	M	2,154		2.0	6.9	29.2	6.9
11*	14	M		700	4.6	11.5	25.2	9.4
12	70	M	447		6.2	6.2	24.1	5.1
13	35	M	440	417	7.2	11.4	29.4	11.4
14*	20	F	1,662	72	5.9	9.0	18.9	5.8
15	14	M		609	5.2	12.0	15.4	6.3
16	21	M	482	189	11.6	11.6	7.8	3.1
17	36	M	1,060	509	12.4	15.0	7.3	3.7
18	17	M	421	109	13.4	13.4	4.4	2.0
19	48	M	160	80	16.1	13.9	3.5	1.7
20	15	F	38	67	14.4	14.4	2.8	1.4
21	16	M	56		18.1	18.1	2.0	1.2

* Mixed *Ancylostoma duodenale-Necator americanus* infection.

TABLE VI

Blood loss per hookworm and relationship between blood loss and number of ova in pure Necator americanus infection

Patient	Fecal blood loss period* A	Fecal blood loss period* B	Number of *Necator* recovered	*Necator* ova period A	*Necator* ova period B	Blood loss	Blood loss
	ml./day			*thousand/day*		*ml.* $\times 10^{-2}$*/ Necator/day*	*ml./*10^4 *ova/ day*
2 (1)	43.17	7.36	2,586	2,983	379	1.38	1.38
2 (2)	7.36	0.96	457	379	75	1.40	2.11
3	96.94	3.47	1,641	6,748	138	5.69	1.41
4 (1)	65.00	8.13	1,023	1,639	147	6.35	3.81
4 (2)	8.13	5.73	98	147	47	2.44	2.40
5	29.02	3.33	1,684	1,708	27	1.53	1.53
6	51.30	8.75	840	3,281	240	5.07	1.40
13	17.87	1.45	417	440	0	3.94	3.73
15	15.40	0.73	609			2.41	
16	7.74	1.84	189	482	0	3.12	1.22
17	7.26	1.23	509	1,060	0.9	1.18	0.57
18	4.41	0.53	109	421	1	3.55	0.92
19	3.49	0.17	80	160	0	4.15	2.08
20	2.74	1.81	67	38	13	1.39	3.72
Average						3.11	2.02
Range						1.18–6.35	0.57–3.73
Standard deviation						±1.73	±1.12

* A = Study shortly before vermifuge; B = Study after vermifuge.

than from the lower bowel cannot be decided but appears quite possible. It is improbable that this radioactivity should represent Cr^{51} which remained suspended outside the injected erythrocytes, since the cells were washed three times before being administered by mouth or stomach tube, and since absorption of radiochromate, when given in water, although apparently significant is also small (Table IV).

Except in one case, there was no detectable radioactivity in the erythrocytes of patients given Cr^{51} into their intestinal tract. It would seem, then, that what little Cr^{51} is absorbed does not tag the circulating erythrocytes.

From these studies it appears that, within certain limits, measurement of fecal radioactivity and its comparison with blood radioactivity are valid ways of estimating the amount of blood lost into the gastrointestinal tract through a bleeding lesion, in patients whose erythrocytes have been marked with Cr^{51}. On the other hand, while studies with radioactive chromium tell us fairly accurately the amount of blood which is lost into the gastrointestinal tract, they give us no information regarding possible reabsorption of the various blood elements from the intestinal lumen.

The total loss of blood and iron per patient

The total blood loss per day may be considerable (Table V), and is roughly proportional to the intensity of infection. To obtain a more exact value in the column indicating fecal blood loss (Table V), one would have to subtract as a "blank" the small quantity of Cr^{51} which would

TABLE VII

Estimate of blood loss in Ancylostoma duodenale infection

Patient	Blood loss*	No. of *Necator*	Probable blood loss due to *Necator*	Probable blood loss due to *Ancylostoma*	No. of *Ancylostoma*	Probable blood loss per *Ancylostoma*
	ml./day		*ml./day*	*ml./day*		*ml.* $\times 10^{-2}$*/day*
1	64.18	2,987	92.90	?	124	?
7	40.07	76	2.36	37.71	182	20.72
8	29.70	453	14.09	15.61	49	31.86
11	16.72	327	10.17	6.55	32	20.47
14	2.09	53	1.65	0.44	9	4.89

* Calculated from formula (*1*).

be excreted by the individual under study if he were not infected by hookworm. Such a "blank," however, appears to be quite variable from patient to patient (Table II) and using the average fecal Cr^{51} found for the 10 non-infected subjects as "blank" does not appear justified.

In patients 1, 2, 3, 4, and 6 who entered the hospital in poor condition, with a low blood hemoglobin, blood loss soon after admission (Table V, column 5) was distinctly greater than after they had been treated with iron and diet, or transfusions. These patients will be reported elsewhere in more detail (17). Conversely, some patients with a rather intense infection bled relatively little. Thus, patient 17, who harbored at least 509 worms, bled 7.3 ml. per day and patient 15, with at least 609 worms, 15.4 ml. per day. Patient 15 entered the hospital with a hemoglobin of 5.2 Gm. per 100 ml., but he was not studied at this time. At the time of study, he had recovered from the anemia and was in excellent state of nutrition. Patient 17, who had no significant anemia on admission, had a hemoglobin of 15.0 Gm. per 100 ml. at the time of study. Contrary to most of the patients in this study, his dietary history was good. Being both a fisherman and a farmer, he ate fish and fresh vegetable twice daily, and drank milk daily.

The data on iron loss in Table V are an approximation only since iron may be in part reabsorbed after it arrives in the intestinal tract. In Venezuela, the average daily iron intake has been estimated to be around 12 mg. per day (18). If it is assumed that of those 12 mg. a maximum of 3 to 4 mg. is absorbed, it is plain that the patients with severe infection are in negative iron balance, with the possible exception of patient 17. It is interesting that only those six patients (from 16 to 21) who had calculated iron losses of less than 4 mg. per day were free from significant anemia on admission.

The loss of blood per hookworm per day

The work of Wells (6) and of Nishi (7) showed clearly that the dog hookworm, *Ancylostoma caninum*, consumed considerable quantities of blood. Wells arrived at an estimate of this quantity by sucking into a calibrated pipette the blood being ejected from the anus of the worms fixed to the intestine of the anesthetized dog, diluting it in known amounts of counting fluid and comparing the red blood count thus obtained with the dog's red blood count. He concluded that, if the same rate of suction which prevailed during the period of observation (approximately 20 minutes) continued during the 24 hours in the intact dog, each hookworm could consume as much as 0.84 ml. of blood per day. Nishi (7) with similar methods, concluded that the blood loss per *Ancylostoma* per 24 hours was on the average "0.144 ml.," and could be as high as "0.392 ml." if the hookworm continued sucking with a maximum activity throughout the 24 hours. Apart from the hypothesis of the constant rate of suction, two objections may be made to this interesting work: 1) it is known that the red blood counting method has a large intrinsic error, and 2) these results would apply only to the dog hookworm, a larger and probably more voracious animal than the human parasite, specially the *Necator*. If we assumed 0.84 ml. per worm as an average daily loss for the human hookworm, some of the patients in the present series would be losing more than two liters of blood per day into their intestinal tract, an improbable figure. Gerritsen, Heinz, and Stafford (19), using radioiron and methods similar to the ones described here, studied three patients with hookworm infection. They did not mention the exact species involved, but they found respectively 0.026, 0.22 and 0.053 ml. per day per hookworm, values much closer to the present ones. It is possible that the higher value of 0.22 may have been due to the presence of *Ancylostoma duodenale*. Since an unknown quantity of the lost iron is possibly reabsorbed, Gerritsen's values are presumably closer to the actual amount of iron lost by the patient, while the present values should give a more exact indication of the amount of blood actually lost into the intestine through the action of the worms.

The amount of blood loss is variable, ranging in the various patients with pure *Necator* infections from extremes of 1.18×10^{-2} and 6.35×10^{-2} ml. per worm per day (Table VI). As for *Ancylostoma duodenale* (Table VII), it would appear from the limited number of patients herein presented that blood loss due to these worms is distinctly larger than that due to *Necator*. The data expressing the probable loss per *Ancylostoma* are of course unreliable, since it is assumed, for

the sake of calculation, that the *Necator* present in mixed infections are consuming a quantity of blood equal to the average in pure *Necator* infection, this parameter being subject to considerable variations (Table VI), and since the percentage of *Ancylostoma* is generally low. In patient 7 (Table VII), the figure is more reliable, since 70 per cent of the hookworms were *Ancylostoma*. It is plausible that *Ancylostoma*, a larger worm, will consume more blood than *Necator*, and 0.2 ml. per day per worm appears to be a fair estimate of its capacity to produce blood loss from the host.

In cases in which the recovered hookworms were placed in a test tube and their radioactivity determined, it was a surprise to find that in none of the tubes was radioactivity above background. This finding would imply that the hookworm contains little or no blood, possibly because they eject it under the action of the vermifuge, or lose it during the washing process. According to Wells (6), a number of observers have seen that specimens of *Ancylostoma duodenale* removed from the host at autopsy eject blood both from the mouth and the anus.

The relationship between number of ova in the stools and blood loss

The amount of blood lost per day per million ova is on the average 2.02 ml. ± 1.12 for pure *Necator* infections (Table VI). Thus, there is a rough correlation between blood loss and egg laying, which is to be expected, since both are expressions of the biology of the worm, and hence should depend upon the number of worms present.

The above method of calculating blood loss, while interesting from a biological point of view, is impractical for the clinician who wishes to gain an idea of the amount of blood his patient is losing from the number of ova which he finds in the stools. Since it will usually be difficult for the clinician to collect feces in four-day periods in a quantitative way, the result of the first three ova counts performed on each patient was expressed also in terms of ova per Gm. of feces, and it was attempted to correlate this value with the amount of blood lost per day during the same period of time. By averaging the three ova counts, it was found that the patient lost on the average 2.74 ml. per thousand ova per gram of stool, with range of 0.82 to 7.14 ml. and standard deviation of ± 1.50. Thus the range is rather wide, depending in part on the presence of *Ancylostoma* in some of the cases, but the method has been found useful in roughly predicting the blood loss, and in judging whether or not an anemia associated with hookworm infection is due chiefly to that infection.

Contribution of blood loss to the anemia of hookworm infection

Whether bleeding due to the hookworm is sufficient to account entirely for the anemia cannot be said from the present data alone. Certainly there appears to be a rough inverse correlation between the amount of bleeding and iron loss and the hemoglobin values. Ultimately, whether a patient with hookworm infection develops anemia or not probably depends on a balance between what goes into the red blood cell and what comes out of the body of the patient. This does not necessarily involve iron intake and blood output alone; factors of protein ingestion, as well as of iron absorption, red cell destruction and possibly bone marrow function may enter the picture. Until these factors are quantitatively studied, no definite conclusion can be reached, although it appears probable that bleeding does play an important, if not the most important, role in the genesis of hookworm anemia.

SUMMARY

1. Two non-infected and six hookworm-infected subjects were given their own chromium-tagged erythrocytes via a duodenal tube. Fecal recovery was on the average 96.7 per cent of administered radioactivity, and urinary recovery 1.7 per cent.

2. In ten non-infected subjects, two of them with marked anemia, whose circulating erythrocytes had been tagged with Cr^{51}, there was little fecal excretion of radioactivity (average 1.27 ml. of "blood" per day). Most of the daily excreted radioactivity appeared in the urine (96.3 per cent of the total activity recovered daily, on the average) in the eight non-anemic subjects.

3. A comparison of the radioactivity in blood and feces of patients with Cr^{51}-marked erythrocytes appears to be a valid way of estimating intestinal blood loss.

4. The circulating erythrocytes of 21 patients with varying severity of hookworm infection were

marked with Cr^{51}, and intestinal blood loss due to the hookworm was measured by comparing stool and blood radioactivity.

5. Blood loss per day in the stool was found to range from 2.0 to 251.5 ml., in rough proportion to the severity of infection.

6. In twelve patients with pure *Necator americanus* infection, the blood loss per day per hookworm was on the average $3.11 \times 10^{-2} \pm 1.73 \times 10^{-2}$ per ml.

7. Five patients were found to harbor mixed *Necator-Ancylostoma* worms. Blood loss per *Ancylostoma duodenale* was estimated to lie in the neighborhood of 0.2 ml. per hookworm per day.

8. Calculated iron loss ranged from 1.2 to 29.1 mg. per day.

9. There was a rough correlation between number of ova in the stools and amount of blood lost. On the average, patients lost 2.74 ml. per day per 1000 ova per gram of stool, with standard deviation of $\pm$ 1.50 and range of 0.82 to 7.14 ml.

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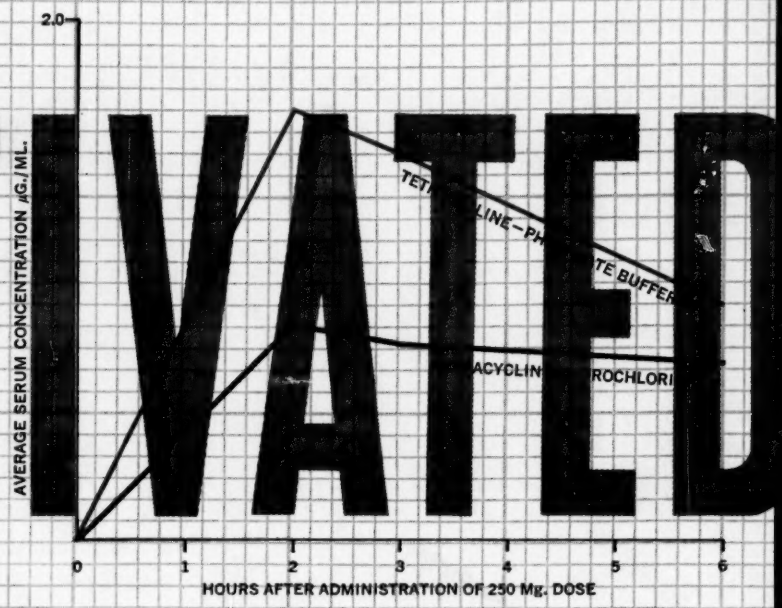

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